Influence of Reflective Mulch on Pinot noir Grape and Wine Quality

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by

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Abstract of a Thesis submitted in fulfilment of the degree of
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A trial established in 2003 at Upper Moutere in Nelson, New Zealand, was used to evaluate the effect of mussel shells as reflective mulch on Vitis vinifera L. cv. Pinot noir vine performance and fruit and wine quality.

Shell mulch had several effects on the environment and vine growth as well as grape and wine composition in the 2006/2007 season. Soil under mulch was cooler compared to un-mulched control, but buffered the extremes in temperatures. Fruiting zone temperature over shells was slightly higher during the day and cooler at night, showing no effect on mean hourly temperature. Shell mulch reflected greater amounts of UV-A, UV-B and PAR radiation into the fruiting zone. Shell reduced weed growth compared to control. Leaf petiole and blade samples showed higher amounts of calcium compared to control. Leaf SPAD values were higher in the shell treatment during veraison, previous and postharvest, but lower post budburst. While date of budburst was not affected by treatments, dates of flowering and veraison appeared to be slightly advanced over shells. Fruit set was similar between treatments and was considered poorer in shell bunches due to a larger population of seedless berries. Vine growth was not affected in terms of the number of nodes laid at pruning, flower cluster and shoot number pre shoot thinning, early shoot growth and lateral shoots development. Vigour was not increased by shells as demonstrated by pruning weights, canopy density and trunk circumferences being similar, though internode lengths in shell shoots were greater in 2007 and lower in 2006. Berry weights, bunch weights and vine yields were lower in shell than control, though greater berry numbers were recorded. There were slight differences between treatments in fruit and wine composition. Grape pH only varied in the middle of the sampling time, being higher the 2\textsuperscript{nd} week and lower the 3\textsuperscript{rd} week in shell grapes and TA was greater at harvest time. However, °Brix was only higher in shell grapes in the middle of the sampling period, being similar to control at veraison and harvest. Peduncle lignification was delayed at veraison as well as at harvest time. Shell must after crushing was greater in Brix but similar to control in pH and TA. Similarly, shell wines pre bottling showed
higher alcohol and no differences for pH and TA. HPLC-DAD analyses of commercial-scale and microvin wines showed consistent differences of the individual flavonoid composition. Shell microvin wines were greater than control in quercetin and resveratrol. However, commercial shell wines were lower in epicatechin, gallic acid, resveratrol, and catechin than control. Leaf phenolic composition was also different between treatments. However, further analyses by HPLC-MS in wines as well as in leaves are necessary to identify individual compounds. Total anthocyanins and total phenolics were no different between treatments. Sensory analyses of microvin and commercial shell wines exhibited consistently lower levels of green and unripe tannins, and greater smoothness and complexity as well. Further analysis by GC-MS and HPLC-MS is warranted. Shell mulch improved sensory characteristics of the resulting wines.

**Key words:** Pinot noir, mussel shell, reflective, mulch, UV radiation, wine phenolics, aroma compounds.
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CHAPTER I
INTRODUCTION

1.1 Pinot noir momentum in NZ

According to the New Zealand Winegrowers Statistical Annual (2006), New Zealand’s wine industry is in a successful growth phase. Exports have grown by 12% in volume and 18% in value, reaching 58 million litres valued at $512 million during the current year. The data indicate that Pinot noir is the second most planted grape variety after Sauvignon blanc, with 3,894 hectares, the second most exported variety with 4,150,853 litres and the third largest harvested tonnage, with 22,062 tonnes during 2006 (Figure 1-1). The planted area of Pinot Noir has increased rapidly, and has become an important component of the New Zealand Wine industry.

![Figure 1-1: Pinot noir area planted 1992-2008 in New Zealand (according to data obtained from New Zealand Winegrowers, Annual Statistics)](image)

1.2 Wine style and quality

Red wine quality and its complexity potential are determined by a balance between colour, tannins, flavours and alcohol level. These factors depend on the harvest time of grapes and are related directly to the development of fruit during the season. In fact, tannins have certain roles in wine quality, contributing to body and mouthfeel (Gawel et al., 2000) and to the colour stability due to the formation of polymeric complexes with anthocyanins (Vidal et al., 2002). Because of this, wine grapes need to be harvested at their optimal levels of maturity for producing high quality wine. An optimal maturity not only consists of an appropriate level of sugar, acidity and pH, but also phenolic
compounds associated with the colour and astringency of red wines. These are accumulated primarily in the skin and seeds of the berry and afterward are extracted into the must and wine during alcoholic fermentation. However, Pinot noir usually presents a lack of colour and a high sugar level at the end of the growing season due to a delay in the harvest as winemakers are looking for less green flavour characters. Unfortunately, the development of flavour and phenolic compounds does not coincide with optimal levels of sugar, acidity or pH (Bautista-Ortín et al., 2006). Consequently, it is necessary to find techniques that allow harvesting high quality fruit improving flavour and phenolic ripeness (less green tannins), but at a lower potential alcohol level.

Several studies have focused on the effect of light and heat on grape composition, and different techniques designed to alter the canopy environment have been used in the vineyard, such as leaf removal, site selection, vine spacing, summer pruning (Coventry et al., 2005), defoliation (Pieri and Fermaud, 2005) and bunch exposure (Downey et al., 2004b). However, some of these management techniques also affect other berry compounds.

The use of reflective mulch could become a way to improve wine grape maturity. Sunlight is important for quality grape production due to its effect on photosynthesis and development of secondary metabolites. Smart and Robinson (1991) suggested that improving the light regime in the cluster zone increased the ripening potential. Indeed, it has been reported that an increase of light energy of at least 20% in the canopy can make the grapes sweeter, less acidic, more colourful and aromatic. At the same time, vines were more productive and less susceptible to diseases (Coventry et al., 2005).

This research seeks to determine the efficacy of using mussel shells as a reflective mulch to improve the balance of ripening on Pinot noir. It is of critical importance in cool climate wine regions as Nelson where the balance of harvesting parameters such as phenols, aroma compounds and alcohol level are a usual challenge. The hypothesis is that the chemical composition and yield components of grapes as well as phenological growth stages would be affected by higher light and temperature coming from reflective shell mulch. Not only it is suspected to obtain an enhancement of quality in grapes under adequate levels of sugar, pH and titratable acidity, but also increases of colour, and desirable aroma and flavour compounds. At the same time, changes on conditions of soil moisture, weed development and nutritional status would be also expected.
2.1 Grapevine and wine phenolics

Phenolic compounds in grapes are essential for the quality of wine because they are associated with red wine colour and involved in oxidative processes of white wines (Cheynier et al., 2006) and also contribute to taste by providing bitter and astringent properties (Kennedy et al., 2001).

Phenols in grapes (*Vitis vinifera* L.) and wine are classified in two groups, flavonoids and the non-flavonoids. Each group is divided into several families which have shared structural features that confer properties such as colour, aroma and taste (Cheynier et al., 2006). The most important classes of flavonoids found in grapes and wine are proanthocyanidins (or condensed tannins) which are present in the highest proportion, followed by anthocyanins, and flavonols present at low levels (Souquet et al., 1996).

Wine phenolic composition depends on the grape quality and on winemaking processes that determine their extraction into the must (Cheynier et al., 2006). While anthocyanins accumulate from veraison in the skin of red grapes during ripening (Boss et al., 1996), flavonol synthesis happens in two periods in the skin (Downey et al., 2003b): at flowering and one or two weeks after veraison during ripening (Downey et al., 2006). Tannin biosynthesis occurs independently in the skin and in the seeds of grapes (Robinson, 2006). In the seeds, tannin synthesis occurs after fruit set, with the highest levels at veraison when seed embryo is fully mature (Downey et al., 2006). However, tannin synthesis is completed before veraison in the skin (Robinson, 2006).

The level of extractable tannins decreases in seeds and skin between veraison and harvest, and this decrease in terms of extractability represents a decrease in the bitterness and astringency of tannins in the grape (Downey et al., 2006).

2.1.1 Anthocyanins

Anthocyanins are responsible for the colour of red wine and grapes (Ribéreau-Gayon and Glories, 1986; Downey et al., 2006). These compounds are located mainly in the thick-
walled hypodermal cells of the skin, the same as where the tannins are found (Adams, 2006), and are also present in the leaves at the end of the growing season (Ribéreau-Gayon et al., 2006b).

There are two chemical forms of anthocyanin dependent on the vacuolar pH: the neutral quinonoid anhydro base and the flavylium cation form. Varieties such as Pinot noir contain only non-acylated forms of five wine grape anthocyanidins: cyanidin, delphinidin, peonidin, petunidin and malvidin (Adams, 2006).

2.1.2 Flavonols

Flavonols act as UV protectants and free radical scavengers (Downey et al., 2003b) and contribute to wine colour as anthocyanin co-pigments (Boulton, 2001). The most important flavonols in wines are quercetin, kaempferol, myricetin and isorhamnetin (Adams, 2006). Quercetin-3-O-glucoside and -3-O-glucuronide are the most prevalent in grape berries (Price et al., 1995), with a concentration of around 0.065 mg/g fresh weight of skin in Pinot noir at harvest. However, quercetin is present in red wine as aglycone (Ribéreau-Gayon et al., 2006b).

2.1.3 Tannins

Grape tannins contribute to the mouthfeel of wines (Gawel et al., 2000) and also provide colour stability due to the formation of polymeric complexes with anthocyanins (Vidal et al., 2002). Tannins are predominantly bulky phenol molecules which produce stable combinations with proteins and polysaccharides (Ribéreau-Gayon et al., 2006b). The main tannins in grape berries are the polymeric flavan-3-ols, which are located in the hypodermal layers of the skin and the soft parenchyma of the seed between the cuticle and the hard seed coat (Adams, 2006). These tannins include oligomers and proanthocyanidin polymers that are composed of monomeric flavan-3-ols including subunits such as catechin and epicatechin (Downey et al., 2006), with 2,3-trans and 2,3-cis molecular configuration, respectively (Adams, 2006). A basic catechin unit may not be considered as tannin because the molecular weight is too low; usually tannins are organized as dimeric, trimeric, oligomeric or condensed tannins which include more than ten flavan units, with a molecular weight higher than 3000 Dalton (Ribéreau-Gayon et al., 2006b). A hypothetical condensed procyanidin is made of four subunits such as
catechin, epicatechin, epigallocatechin, which are referred as extension subunits, and epicatechin gallate, which the terminal unit (Adams, 2006).

Previous studies have observed high amounts of tannins present in seeds and skins at veraison, at the onset of ripening (Kennedy et al., 2001). However, the biosynthesis of tannins from flavan-3-ols is still not clearly understood because it is unknown how and where interflavan bonds are formed (Adams, 2006). The results found in the published literature suggest that tannin synthesis would occur prior to veraison in the seeds and its accumulation is independent of that in the skin. An early accumulation of tannins in the skin would be related to early expression of genes involved in the flavonoid biosynthetic pathway (Downey et al., 2003a).

Skin tannins and seeds tannins are different from each other; while skin tannins have a higher degree of polymerization than seed tannins, the latter usually present a higher proportion of their subunits as epicatechin gallate. This is in contrast with skin tannins, which contain mainly epigallocatechin subunits (Adams, 2006). This difference has been used to define proportions of both seed and skin tannins in Pinot noir wines (Peyrot des Gachons and Kennedy, 2003).

Other important phenolics in berries are classified as non-flavonoids, which are the hydroxycinnamates present mainly as trans-isomers in hypodermal cells of the skin and in mesocarp cells of the pulp (Adams, 2006), as well as stilbenes which are produced by the fruit and vegetative tissues in response to fungal infection and exposure to high levels of UV light (Jeandet et al., 1991). The normal flavonoid metabolism is diverted towards stilbenic derivatives such as trans-resveratrol and its glycoside by the action of the enzyme stilbene synthase (Ribéreau-Gayon et al., 2006a). This resveratrol is extracted into the wine during fermentation and appears to have beneficial properties for health (Ribéreau-Gayon et al., 2006b).

### 2.2 Influence of viticultural management on grape and wine phenolics

Flavonoid biosynthesis is affected by many factors, with light and temperature being the most important, but also including altitude, soil type, water, nutritional status, microbial interactions, pathogenesis, wounding, defoliation and plant growth regulators (Downey et al., 2006).
Some effect of nutrients on anthocyanins amount has been reported. Colour in grape berries decreases with low as well as high levels of nitrogen fertilizer (Delgado, 2004), and through high levels of potassium (Jackson and Lombard, 1993).

The influence of vine vigour on flavonoid content in Pinot noir grapes was investigated by Cortell et al. (2005), who measured high proanthocyanidin accumulation, especially epigallocatechin, with decreasing vigour and related these results with increases in light and/or heat exposure in the canopy. However, in relation with these results, Downey et al. (2006) suggested that it is not clear whether this change is related with differences in vine vigour or is an effect of bunch exposure.

Regarding irrigation, some reports suggest that water stress increases tannins and anthocyanins in grapes (Dry et al., 1998). Other research shows that excessive irrigation decreases tannin content and that water deficit would not have an effect on tannin or anthocyanin accumulation (Kennedy et al., 2000). It is clear that water deficit causes a decrease in berry size, changing the ratio of skin weight to total berry weight and thus anthocyanin and tannin concentration in the berry (Downey et al., 2006). It has been suggested that changes in the structure and development of skin, especially in the inner mesocarp tissue, are responsible for changes in phenol concentration, rather than any direct effect of them on flavonoid biosynthesis (Roby et al., 2004).

Although, the amount and type of flavonoids synthesised in grapes have been influenced by climatic conditions and viticultural management during the growing season, the nature of these interactions is not yet known (Robinson, 2006).

Smart et al. (1988) described that shading decreased flavonoid content and carbon fixation, resulting in lower levels of sugar and organic acids in the fruit. In addition, shading alters temperature and humidity within canopies (Haselgrove et al., 2000). An increase of the humidity produces lower levels of vapour pressure deficit, transpiration and photosynthesis, reducing growth and flavonoid accumulation; moreover, it can increases the risk of fungal or bacterial infection (Downey et al., 2006), which may induce the synthesis of phytoalexins in response to Botrytis cinerea. Phenolic compounds are affected by oxidation due to laccase enzyme which is produced for these fungi (Ribéreau-Gayon et al., 2006a). Furthermore, low temperatures decrease the rate of
metabolic processes and accumulation of metabolites. Temperatures above 30°C can also stop or reduce the rate of some metabolic processes (Downey et al., 2006).

There are several reports that explain the effect of sun exposure on grape composition, particularly phenolic and flavonoid compounds, and the relationship between shading and grape colour.

Generally, low light reduces colour in Pinot noir (Kliwer, 1970), Shiraz (Smart et al., 1985), Cabernet Sauvignon (Dokoozlian and Kliewer, 1996). It has been created a consensus that low light reduces anthocyanins, while high light increases flavonoid content. However, some studies have not observed changes in total anthocyanins in response to shading, whereas a decrease in anthocyanin levels has been reported under high light (Spayd et al., 2002). On the other hand, Downey et al. (2004a) obtained differences in anthocyanin composition, but not in the total amount. These contradictory results could be explained by factors such as cultivar, site and season, which can dramatically affect the flavonoid content (Downey et al., 2006).

Other authors have observed anthocyanin differences depending on which side of the canopy was sampled. Bergqvist et al. (2001) examined north and south facing canopies in the northern hemisphere in Cabernet Sauvignon and Grenache berries and observed that anthocyanins increased with increasing light up to 100 µmol/m²/s on the shaded side (north), but they decreased when exposure exceeded this amount on the sunny side (south). Therefore, berry composition would be influenced by direct (light quantity and quality), and indirect effects (temperature) of sunlight exposure. On the other hand, Spayd et al. (2002) studied the east-west exposure in Merlot berries and obtained higher levels of anthocyanins from the east side of rows, which was exposed to lower temperatures (morning sun).

It is difficult to separate the effects of light and temperature on anthocyanin biosynthesis. Both parameters are highly interactive because the temperature is determined by the flux density of absorbed radiation and convective heat loss from the vines (Smart and Sinclair, 1976). Spayd et al. (2002) studied the effect of light and temperature separately; while heating the less exposed fruit obtained lower anthocyanin concentration, cooling the high exposed fruit on the west side increased the level of anthocyanins, concluding that the accumulation of anthocyanin is more a function of temperature than of light.
Similar results were observed by Downey et al. (2004a) who compared the anthocyanins from shaded fruit in two different seasons, and obtained lower anthocyanin levels during the hottest season. Moreover, it has also been observed that lower night temperatures produce higher anthocyanin accumulation (Mori et al., 2005).

Both light and temperature affect anthocyanin composition. Higher proportions of trihydroxylated anthocyanins (Downey et al., 2004a) and a glucoside substitution to coumaroyl-glucosides (Haselgrove et al., 2000) have been reported with shading. Some effects of temperature on compounds such as coumaroyl glucosides and derivates have also been reported (Downey et al., 2004a; Spayd et al., 2002). Furthermore, Downey et al. (2006) suggested that differences in anthocyanin composition between warm and cool climates were related to the temperature, but it is not known if the reduction of colour in berries observed in very hot seasons is due to degradation of anthocyanins or to reduced anthocyanin biosynthesis.

The effects of light and temperature on other flavonoid components have also been studied. The amount of grape flavonols is dependent on light exposure of the tissues where they are accumulated in varieties such as Pinot noir, Syrah and Merlot (Adams, 2006). Moreover, higher levels of flavonol glucosides have been found in exposed fruit, or even in individual berries from the same side of the cluster in Pinot noir (Price et al., 1995). It has also been reported that the levels of flavonols in leaves as well as fruit decrease when the tissues are shaded (Downey et al., 2004a).

On the other hand, bunch exposure has not affected the tannin accumulation in Shiraz grape berries. At the same time, it has been observed some effect of light on proanthocyanidin content and composition, in seeds as well as in skins (Downey et al., 2004a). However, a report indicates that proanthocyanidin content and composition during berry development would be affected in shaded and exposed fruit, being lower in shaded fruit (Downey et al., 2006). The tannin extractability decreased in both shaded and exposed fruit, but the concentration was higher in exposed fruit.

In general, the most important influences of flavonoid content are site and season (Guidoni et al., 2002).
2.3 Aroma compounds

2.3.1 Definition of aroma

According to Rapp (1998) and Ribéreau-Gayon et al. (2006b), there are four groups of wine aromas: primary or grape aroma derived from the grape berry metabolism; secondary aromas formed during the processing of grapes and chemical reactions in the must, during extraction of the juice and maceration; compounds developed during alcoholic fermentation and the last group which includes compounds obtained from chemical and enzymic reactions during the aging of the wine. However, Clarke and Bakker (2004) categorized as secondary aroma compounds those which come from the processes happening in grapes and during the fermentation, and as tertiary aromas the compounds related with maturation and aging.

2.3.2 Grapevine aroma components

Aroma compounds are contained in grape skins, they are specific to certain varieties and represent the aroma potential in grapes and wine (Galet, 2000). The potential aroma of grape derives from aromatic free volatiles and from odourless precursors (bound or non-volatile), which can be hydrolysed during the winemaking process (Arévalo et al., 2006).

2.3.2.1 Terpenes

Primary wine aroma is mainly due to the presence of monoterpenes, which are present in various grape varieties (Pisarnitskii, 2001). Several studies have shown that these compounds are located in skin of berries, are linked to sugar, and play a significant role in the varietal flavour of wines (Mateo and Jiménez, 2000).

Total free monoterpane concentration classifies the varieties as flavoured muscats with high concentrations up to 6 mg/l; non-muscat but aromatic with a concentration of 1 to 4 mg/l; and neutral when varieties do not depend on monoterpenes (Mateo and Jiménez, 2000).

Mateo and Jiménez (2000) define three types of monoterpenes in grapes: free aroma compounds, commonly dominated by linalool, geraniol, nerol, citronellol and \( \alpha \)-terpineol (Pisarnitskii, 2001) also known as free volatile terpenes (Winter, 2002);
polyhydroxylated forms or free odourless polyols which do not make a direct contribution to the aroma, but can give strong volatiles depending on pH and temperature (Galet, 2000); and glycosidically conjugated forms, which represent the majority of terpenes and are also known as potentially volatile terpenes. In general, most grape varieties contain free and bound glycoside terpenes (Mateo and Jiménez, 2000) and the combination of the aroma of each compound determines the wine bouquet (Clarke and Bakker, 2004). In fact, geraniol has been found in Pinot noir wines contributing to floral and cherry flavours (Fang and Qian, 2005).

2.3.2.2 Carotenoids (C13 - norisoprenoid precursor)
Carotenoids are accumulated during the ripening of grape berries in the skin and their oxidation produces C13-norisoprenoids, which are strongly odoriferous (Pisarnitskii, 2001).

These compounds are divided into two main forms, megastigmanes (oxygenated) and non-megastigmanes (Ribéreau-Gayon et al., 2006b). Norisoprenoid compounds include β-ionone (viola scented) and damascone (aromas of exotic fruits), with threshold concentrations of 7 and 9 ng/l, respectively; β-damascone (rose and fruits), β-ionol (fruit and flowers), 3-oxo-β-ionone (tobacco) are others norisoprenoids present in grapes, but in lower amounts (Pisarnitskii, 2001). Although β-damascenone is present in all grape varieties (mainly in red wines), only in some cases it has an impact on global aroma (Ribéreau-Gayon et al., 2006b). However, β-damascenone has been cited as an important odour-active compound in Pinot noir (Fang and Qian, 2005; Miranda-Lopez et al., 1992), Syrah and Cabernet Sauvignon (Ristic et al., 2007; Joscelyne et al., 2007).

2.3.2.3 Methoxypyrazines
This family of volatile compounds is present in green plant parts, including green berries (Winter, 2002). The most important methoxypyrazines occurring in wine grapes are 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-sec-butylpyrazine and 2-methoxy-3-isobutylpyrazine which exhibit vegetative or herbaceous aroma to wine, such as green pepper, asparagus and even earthy notes (Ribéreau-Gayon et al., 2006b).

Methoxypyrazines contribute to the flavour of white wine at low level (Allen et al., 1991), mainly in Sauvignon blanc. However, this compound appears to be much higher in red wine varieties such as Cabernet Sauvignon, Cabernet Franc and to a lesser extent,
Merlot (Roujou de Boubée et al., 2000). Although some cultivars such as Pinot Noir contain the precursor 2-hydroxy-3-isobutylpyrazine, 2-methoxy-3-isobutylpyrazine is not found in their grapes or wine due to a low level of O-methyltransferase activity (Hashizume et al., 2001). It has been found in Pinot noir that 30 days after anthesis the 2-methoxy-3-isobutylpyrazine level in grapes were 1.4 ng/L and it dropped to below 0.2 ng/L at harvest (Hashizume and Samuta, 1999). In fact, 2-methoxy-3-isobutylpyrazine decreases naturally after veraison, which is determined by the amount of berry exposure and is associated to the decrease in malic acid (Roujou de Boubée et al., 2000). At harvest time, it has been described that stems contain about 53% of the 2-methoxy-3-isobutylpyrazine, seeds 15%, skins 31% and 0.6% in the flesh of a grape bunch (Roujou de Boubée et al., 2002).

2.3.3 Pinot noir wine aroma

The presence of terpenoids and C13-norisoprenoids at various ratios in grape varieties defines different odour descriptions contributing to the formation of secondary wine aroma (Pisarnitskii, 2001). Wine aroma is defined by the combination of terpenoids, C13-norisoprenoids and fermentation products such as ethanol, alcohols, ethers, acids and aldehydes which depend on conditions of the fermentation and yeast strains (Pisarnitskii, 2001).

Fruit aromas such as stone fruits (plum and cherry), strawberry, raspberry, blackcurrant and blackberry have been reported in the aromatic profile of Pinot noir. Around 800 volatile compounds are reported as occurring in Pinot noir wines, but most of them are not odour active. The published literature indicates that there is not a single compound that characterizes the aroma of Pinot noir (Fang and Qian, 2005).

Several compounds that have been identified as contributing to the typical aroma of Pinot noir, such as ethyl and methyl vanillate, acetovanillone and 3-methylthio-1-propanol, along with 3-methylbutanoic, hexanoic, octanoic and decanoic acids, 2-phenylethanol and benzyl alcohol (Brander et al., 1980; Miranda Lopez et al., 1992). In addition, ethyl anthranilate, ethyl cinnamate, ethyl 2.3-dihydrocinnamate and methyl anthranilate have also been reported (Moio and Etievant, 1995; Aubry et al., 1997). According to Fang and Qian (2005), 2-phenylethanol and 3-methylbutanol are important in Pinot noir aroma. In
addition, acids, alcohols, sulphur compounds, esters, aldehydes could be important to characterize the aroma of this variety.

2.3.4 Identification of aroma compounds

Gas chromatography/mass spectrometry analysis (GC-MS) determines chemical structure of volatile compounds. This method can detect glycosides and their aglycones or free volatile terpenes (Mateo and Jiménez, 2000). This technique has been used successfully to identify and quantify grape aroma compounds (Cabrita et al., 2006), the contribution of some compounds to the aromas of white wines (Tominaga et al., 2000) and wine aroma compounds in red wines such as methoxypyrazines (Roujou de Boubée et al., 2000) and odorant compounds (Aznar et al., 2001).

Gas chromatography/olfactometry analysis (GC-O) allows finding important odorants and quantifies their contribution to the global aroma. The volatile fraction of wine can be composed of different compounds and some of them can be odour-active. It is necessary to relate each concentration with a sensorial perception (Aznar et al., 2001). Several olfactometric techniques have been used to determine odour active compounds in wine, including aroma extract dilution analysis (AEDA), Charm analysis and OSME analysis (from the Greek word meaning smell) (Guarrera et al., 2005). Both Charm analysis and AEDA are based on the sequential dilution of an aroma concentrate. In Charm analysis, sensory character and the aroma duration of compound coming from a GC column are recorded, and the importance of some individual aroma to the overall aroma profile is defined by the time and intensity combination (Acree et al., 1984). However, in AEDA method, the aroma profile is sequentially diluted and recorded, and the maximal dilution value at which the aroma compound is detected from the GC effluent is used to calculate the flavour dilution value (Fang and Qian, 2005). A faster analysis is known as OSME technique, where an aroma extract is injected into a GC, and the aroma description and intensity of each peak are recorded through higher values or OSME numbers (Miranda-Lopez et al., 1992).

In particular, the flavour impact of the odorants has been evaluated by extract dilution sniffing analysis using Charm analysis (Moio and Etievant, 1995) and AEDA has been used to obtain a hierarchical list of odorants (Fang and Qian, 2005).
2.4 Influence of viticultural management on grape and wine flavour and aromas

There are several factors cited by Johnstone (2004) that influence the wine flavour such as genetic make up, climate, soil and topography, clone, rootstock and the design of the vineyard.

Some studies have demonstrated that monoterpene aroma compounds are influenced by climate (Rapp, 1998; Reynolds and Wardle, 1997). While grape growing areas located in cool climates produce higher monoterpenes than those in warm climates, norisoprenoids are accumulated more quickly during wine aging when grapes come from a warm climate (Rapp, 1998). On the other hand, the bound fraction of linalool presents a higher concentration in cool climates, and greater floral aroma, whereas the free fraction of linalool is higher in warm regions with high altitude (Winter, 2002).

Winter (2002) suggests that light and temperature are important for aroma development because both drive leaf photosynthesis and sugar production, and they also determine the activity of enzymes. Reynolds and Wardle (1997) studied the effect of bunch exposure on monoterpenes in Gewurztraminer and found that fully exposed fruit had higher free and potentially volatile terpenes than partially exposed and fully shaded fruit. These compounds were affected by different light conditions and potentially volatile terpenes were more responsive to viticultural variation than free volatile terpenes. These results were confirmed under organoleptic evaluation. Some experiments in Canada have shown a fine line between optimal exposure and overexposure, because monoterpenes decrease with too much exposure (Reynolds et al., 1996). This decrease could be caused by the destruction of some molecules through UV radiation, associated with overexposure (Winter, 2002).

Changes on the canopy density promoted by some viticultural practices also have indirect effects on monoterpenes. Hedging and basal leaf removal increased free and potentially volatile terpenes levels in early season cultivars such as Gewurztraminer. At the same time, canopy division, basal leaf removal and amplified vine spacing increase volatile terpenes in Riesling (Reynolds and Wardle, 1997). In fact, leaf removal is a common practice which has an effect on monoterpenes levels in cool climate (Winter, 2002).
Another practice with positive results in monoterpenes has been the reduction of bunch numbers to one per shoot in Shiraz after berry set, improving potentially volatile terpenes at harvest (Bureau et al., 2000). Furthermore, the increase of leaf/fruit ratio enables an earlier ripening, improving the final sugar concentration in berries. However, monoterpene concentrations are not related directly to sugar concentration and yield (Winter, 2002).

Harvest timing is another way to improve monoterpene concentrations. In cool climate conditions, late harvest of Gewurztraminer obtained higher amount of monoterpenes compared to earlier harvest (Reynolds et al., 1996). In general, late harvest has been beneficial in cooler seasons (Winter, 2002).

On the other hand, light has also a positive effect in the norisoprenoid content. The blue-green part of the light spectrum, applied during early ripening, increases carotenoid and norisoprenoid potential (Bureau et al., 1998). It was also observed an advanced carotenoid breakdown and formation of norisoprenoids in Riesling (Marais et al., 1992) and Shiraz (Bureau et al., 2000). However, the concentration of these compounds at harvest decreases in both light and shade exposure, mainly in hot years (Winter, 2002).

Altitude and UV exposure are also affecting norisoprenoids. For example damascenone concentration was higher in cooler years when the altitude is high; whereas these compounds decrease when they are exposed to UV light during ripening (Winter, 2002). However, increasing leaf/shoot ratio by partial crop removal after berry set has not increased norisoprenoid compounds (Bureau et al., 2000).

Methoxypyrazine concentration in grapes and wine is determined by several environmental factors such as climate (Lacey et al., 1991), temperature and sunlight (Hashizume and Samuta, 1999), rainfall, soil composition and moisture (Roujou de Boubée et al., 2002). While warm climates increase 2-methoxy-3-isobutylpyrazine degradation, rainfall and high soil moisture promote high levels (Chapman et al., 2005). Moreover, some viticultural factors contribute to low 2-methoxy-3-isobutylpyrazine levels at harvest, such as leaf removal (Hashizume and Samuta, 1999) and adequate pruning system, which favour degradation and reduce incidence of vegetative flavours and aromas in wines (Allen and Lacey, 1993). In fact, light exposure would promote the
formation of 2-methoxy-3-isobutylpyrazine in immature berries and photodecomposition in ripening grapes (Hashizume and Samuta, 1999).

2.5 Use of Mulches in viticultural management

Several kinds of mulch of different origins have been used in vineyards looking for effects on the productivity and fruit quality of grapevine. Organic mulches include bark, compost, leaves, straw and sawdust. Inorganic mulches are stones, gravel and plastic.

Mulching has a positive effect on the environment of the vineyard. Agnew and Mundy (2002) established several benefits of organic and inorganic mulches: higher soil moisture retention provides a delay in the onset of irrigation; greater soil nutrients levels reduce fertiliser application; soil temperature buffering improves root development and budburst. At the same time, mulching improves weed control; increases soil organic matter and fungi population in the soil, and decreases the bulk density of soil and changes to yeast available nitrogen in grape juice. However, same authors have described significant changes in worm population which express biological activity into the soil, yield, juice °brix and titratable acidity, vegetative growth, pruning weights and petiole or leaf nutrient content (Agnew and Mundy, 2002). In these cases, the mulches were made from vineyard prunings, grape pressings, green waste, pine bark and mussel shells.

Other authors, such as Penfold (2004), suggest positive effects on moisture conservation and weed control, especially using cereal straw and grape stalks. In addition, benefits such as improved vine nutrition and moisture availability may result in higher yields. Watson (2006) experimented that the yield increased almost 40% using compost and straw in some trials which were developed in Australia. Creasy et al. (2003) found effects of mulch on higher weight pruning using black plastic, cocomulch and compost, and suggest stones as another material which can reflect light during the day and radiate heat at night into the canopy.

2.5.1 Reflective mulches and previous research

Reflective mulches have been tested in different crops. Costa et al. (2003) found that they promoted a higher photosynthetic rate, increasing fruit weight and yield in kiwifruit; Bertelsen (2005) obtained a positive influence on flower bud formation and fruit size in
pear. On the other hand, higher yield and colour in tomato and lower aphid populations were found with the use of reflective mulch (Yoltas et al., 2003). Reflective mulch was also tested in persimmons and kiwifruit by Thorp et al. (2001), who obtained an advance in the date of harvest maturity and higher fruit size in persimmons, and an increase in flowering and yield in kiwifruit. Finally, there was an experiment in apples where the treatment produced an increase in fruit number and weight at harvest and incident light available in the canopy (Grout et al., 2004). These studies suggest that the light and temperature produced from reflective mulches are important in fruit development.

According to previous research, reflective mulches have been used in grapevines and some impacts on grape composition and wine quality have been found. Robin et al. (1997) described positive effects on vine productivity, sugar and phenolic compounds of berries as well as sensorial properties of wines using aluminium sheets laid on the ground. Additionally, Spring (2004) achieved effects within canopy, mainly on thermal microclimate of the cluster, without effects on vine productivity. However, in spite of obtaining higher sugar content and lower malic acid content solarising berries by clothes which reflect light, no differences were observed in the composition and taste of the wines made from them. Moreover, lower acidity and higher contents of sugar and free amino acids as well as better colour were obtained in solarised table grapes clusters (Sauvage et al., 1998).

Other authors as Yokotsuka et al. (1999) have obtained some positive effects on the maturation of berries by modifying certain soil characteristics through the addition of oyster shells into the soil. Both sugar level and accumulation of anthocyanins increased in berry juice of Cabernet Sauvignon and Merlot. However, no effects of reflective mulches on anthocyanins and phenolics compounds under increases of light have been found in Merlot and Pinot noir (Vanden Heuvel et al., 2007; Merwin et al., 2005). At the same time, other studies have shown no effect of exposure to full light on berry anthocyanin (Hannah et al., 2004; Price et al., 1995).

Some effects on flavour and aromas have also been found. This was the case with Razungles et al. (1997), who obtained higher amounts of carotenoids, precursors of C13-norisoprenoids, on grapes grown on a type of light reflecting soil.
Findings from a recent study suggest that the major effect of mussel shell mulch used as reflective mulch was related to changes in soil moisture and the light and heat available under the canopy. Soil temperature under shells was between 0.9 and 1.4°C cooler and the soil moisture was greater, especially in the first 30 cm depth. The canopy received four times more reflective UV-B radiation from shells under clear conditions and its temperature was warmer than the control by 1°C for 7.1 hours per day pre-veraison, but not during post-veraison period (Crawford, 2006). These differences mean 804 growing degree hours higher in Shell area than Control over the whole season, which contribute to changes in fruit composition (Creasy et al., 2006a). In addition, there was no effect on vine vigour of the vines in terms of canopy density, but higher pruning weights were found in Shell treatment (Crawford, 2006).

It has been also suggested that there were a relative advancement at the beginning of ripening, but without changes in the composition of grapes and must at harvest in terms of the chemical analysis. Only YAN was higher in the shell treatment (Creasy et al., 2006a).

Yield components have been also affected by increases of light and temperature. Creasy et al. (2006a) reported higher cluster number, but lower yield, due to reduction in berry weight as a result of the shrivelling of berries, especially during the later phase of ripening in one year of the trial.

2.6 Aims and objectives

The literature shows that artificial reflective mulches have been placed in specific growth stages in grapevines, achieving effects on vine environment, composition of grapes and wine aromas. However, no study has been conducted involving a permanent influence during several continuous seasons considering different conditions of weather and vine growth.

The aim of this study was to verify the effectiveness of using mussel shells as a natural reflective mulch to improve the balance of ripening on Pinot noir. First of all, it was suspected to obtain an enhancement of quality in grapes and must under adequate levels of sugar, pH and titratable acidity as well as increases of colour, desirable aroma and flavour compounds, and flavonols and anthocyanins contents in the resulting wines.
Sensory characters of wines were analysed by tasting assessment limited the analysis to aroma compounds (Crawford, 2006).

Another objective was to examine the effect of Shell mulch during periods of budburst, flowering, fruit set, veraison and ripening. Previous results were not clear about if there was an effect of Shell mulch on phenological stages or just seasonal conditions of weather and vine growth. Budburst was not different in two areas of the trial (upper and lower) and both flowering and veraison were more advanced in the shell area (Crawford, 2006).

The third objective of this trial was to examine the effect of higher light and temperature in Shell area on vine growth, vigour and yield components. Crawford (2006) obtained no differences on vine vigour and different trends of growth on the upper and lower areas. Differences in berry weights were not significant between treatments and yield varied with different seasons.
3.1 Trial Site

This study was conducted in a seven year old commercial *Vitis Vinifera* L. cv. Pinot noir vineyard (clone 5 grafted on 101-14 rootstock) located at Neudorf Vineyards in Upper Moutere within the Nelson Winegrowing area. Soils are predominately heavy clay topsoils over gravel subsoil. Vines were vertical shoot position (VSP) trained to two canes on a north-south row orientation and pruned to 20 buds, and planted at a spacing of 1.69 metres (within row) and 2.2 metres (between rows) with 2,683 vines per hectare.

The trial assessed during 2006/2007 season consisted of two treatments each of nine rows. Treatment one was the control, a 1.2 metre wide herbicide strip in the under vine area, and treatment two was a 1.2 metre wide strip of crushed mussel shells in the same area. A continuous strip of mussel shells had been placed in the treatment area in November 2003.

Figure 3-1. Trial site located at Neudorf Vineyard. Green colour represents shell rows and blue colour, the control rows.

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Bottom of hill - North side
Prior to their application, shells had been processed at the mussel factory and then allowed to weather for about six months. After that, shells suffered natural crushing during loading, transport, and application. Six continuous rows of fifteen vines per row from mid-portion of the slope were defined as the experimental area in both treatments (Figure 3-1) due to high level of variation up and down the vineyard slope (Crawford, 2006).

3.2 Trial management

The vineyard was managed commercially under Sustainable Winegrowing New Zealand programme (SWNZ) as practiced by Neudorf Vineyards. Pruning was done from 22nd to 29th June 2006 in the experimental blocks. Crop thinning was not applied during 2006/07 season, but a small number of excessively green bunches were removed after veraison. Multiple row netting was maintained from veraison to limit bird damage. Shoot trimming was done only once, on the 15th January, because the growth was not strong due to cold temperatures in December and overcast conditions in January. Vigorous lateral shoots were trimmed back in the top quarter of the canopy.

3.3 Statistical analysis

During the 2006/2007 season, a number of variables were recorded from both treatments. Some data were collected on a row by row basis, taking measurements from each vine, such as nodes laid at pruning and shoot counts, trunk circumferences, leaf chlorophyll content, flower cluster counts, progression of flowering and colour change through veraison and peduncle lignifications progression, which could help in determining any trends within the blocks (R. Sedcole, personal communication). These data were analysed by comparing means and standard deviations between the shell and control treatments, and relating the mean obtained for each row with the total average of all samples taken. Significant differences were calculated at a 95% confidence interval at \( p \leq 0.05 \). However, a Chi-square test was used to process weed data.

General analysis of variance was applied to analyse grape composition and yield components on whole clusters. While the variability in the clusters was analysed by the two-sample T-test, leaf and wine samples processed by HPLC were performed using
two-way analysis of variance (ANOVA). All statistical analyses were performed using GENSTAT for Windows, version 8.2 ©2005.

3.4 Data collection and analysis

3.4.1 Grape quality according to chemical properties and yield components

Basal clusters from each of the two treatments were collected at harvest 2006 by Dr. Glen Creasy, cooled with ice during transport and then frozen at -20°C until processing after harvest. Ten whole-cluster samples from both treatments were individually assessed for cluster weight, wing weight, rachis weight, berry number, cluster °Brix, pH and titratable acidity (to a pH 8.2 end point). Berries were homogenised using an Ultra-Turrax Homogeniser into a mixture of pulp, skin and seed. Red pigments (anthocyanins) in mg per berry and mg per gram berry weight, and total phenolics in absorbance units per berry and absorbance units per gram berry weight, were measured from a sub sample of the homogenate. Five mL of 50% v/v aqueous ethanol adjusted to pH 2.0 was added to approximately 0.5g of homogenate. This solution was mixed inverting tubes by hand every 10 minutes over a period of 1 hour, after which the tube was centrifuged (3000 x g) for 5 minutes in a Megafuge 1.0 Heraeus (Thermo Fisher Scientific Inc., Waltham, USA). A 0.5mL aliquot of the supernatant was mixed with 5mL 1M HCl; the remaining extract volume was recorded. After 3 hours, absorbance of the diluted HCl extract was read at 700, 520 and 280 nm. The mass of anthocyanins was calculated using a value for the molar extinction coefficient of malvidin-3-glucoside of 30,000 (Wellmann et al., 1976).

3.4.2 Berry variability within the cluster

Ten additional basal clusters collected by Dr. Glen Creasy from each treatment were analysed for cluster yield components, taking cluster and wing weight. Afterwards, berries were separated from the cluster rachis and berry numbers, berry weight and rachis weight were recorded (berries on wings were not analysed). The diameter of 15 berries of a range of sizes per cluster was measured by calliper, to allow the construction of a berry weight to diameter relationship.
For individual berry composition, °Brix (Atago PR-1) was taken for all berries of sufficient size to obtain enough juice to analyse. Malic acid concentration was measured on a berry by berry basis through the use of a reflectometer Merck RQflex (Vallesi and Howell, 2002; Gurban et al., 2006), on ten berries per cluster using a malic acid test kit (Merck, New Jersey, United State).

The red pigments (anthocyanins) were extracted according to the method described by Iland et al. (2000), with a slight modification: skins from individual berries were separated manually from the pulp and seeds and a number of 5 mm skin discs, depending on the berry size, were collected from the both sides of each berry using a cork-boring tool (Yamane et al., 2006). After that, fresh berry skins were macerated in 5 mL of 50% v/v aqueous ethanol adjusted to pH 2.0 for one hour, shaking the tubes to get the contents mixed about every 10 minutes. After a period of one hour, 0.5mL of the supernatant was mixed with 5 mL 1M HCl and after another 3 hours, the absorbance of the extract was read at 700, 520 and 280 nm using a Unicam UV4-100 spectrophotometer (Thermo Electron Corp., Waltham, USA).

Total anthocyanins in mg per berry, mg per gram berry weight and mg per unit berry surface area, and total phenolics in absorbance units per berry and absorbance units per gram berry weight, were calculated from these samples using a value for the extinction coefficient of malvidin-3-glucose of 30,000 (Wellmann et al., 1976). Berry diameter was taken from 15 berries per cluster and then correlated to berry weight, obtaining a formula. After that, the ratio for all of berries per bunch was calculated according to berry weights. Finally, the surface area of the berries was calculated assuming berries were spherical.

3.4.3 Vine growth

3.4.3.1 Budbreak evaluation:
Detailed information on bud break was recorded by Neudorf Vineyard staff on three dates (22nd and 29th September, and 9th October), according to the classification of Eichorn and Lorenz (1977), describing different stages of grapevine growth. Bud burst was considered to have occurred when first leaf tissue was visible (Coombe, 1995).
3.4.3.2 Shoot growth measurements:
In November 2006, shoot and cluster numbers were recorded before and after shoot thinning on 180 vines from the trial site. A cluster was defined as one containing more than 20 flowers and to be on the primary shoot when referring to yield assessment. Shoot lengths and node number counts per shoot were measured the end of November, taking two shoots per vine, one from each mid-portion of the cane. The numbers of laterals shoots produced and their lengths were assessed in one bay per experimental row (30 vines) before leaf plucking from 30th November to 5th December 2006. Measurements were taken from individual shoots vine by vine in the bay, recording numbers and lengths of any laterals that arose from node positions 1 through 4.

3.4.3.3 Trunk circumference:
The trunk cross-sectional area was calculated from trunk circumferences measured at the beginning of November 2006 and at pruning stage (September 2007) from approximately 10 cm above the graft union, corresponding to the first internode. This was done for all vines in the experimental area.

3.4.3.4 Progression of flowering:
The basal cluster of one shoot on each vine was scored visually for the progression of flowering from 25th November to 13th December 2006, using the ranking schedule shown in Table 3-1. The percentage was estimated counting the number of cap-fall in each cluster every two days during flowering stage.

<table>
<thead>
<tr>
<th>Rating</th>
<th>% Fruit flowering / veraison</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0-4</td>
</tr>
<tr>
<td>2</td>
<td>5-15</td>
</tr>
<tr>
<td>3</td>
<td>16-30</td>
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<tr>
<td>4</td>
<td>31-50</td>
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<tr>
<td>5</td>
<td>51-70</td>
</tr>
<tr>
<td>6</td>
<td>71-85</td>
</tr>
<tr>
<td>7</td>
<td>86-95</td>
</tr>
<tr>
<td>8</td>
<td>96-99</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
</tr>
</tbody>
</table>

3.4.3.5 Fruit set assessment:
Fruit set determination was carried out by bagging inflorescences retained on the vine with a small net bag, choosing randomly 6 basal clusters per row, on 25th November
2006. These were collected on 21st February 2007, frozen and then dried in the oven at 65°C for four days in June. Samples were processed in July counting the number of caps as well as the number of berries according to their size for each bunch. Berries were classified into three categories of diameter: seeded (≥ 6 mm), seedless (5 to 4 mm) and shot berries (≤ 3 mm). After that, the fruit set rate was calculated as the ratio of berries over flowers dividing the number of calyptra per inflorescence by the total number of berries per bunch (Lebon et al., 2004).

3.4.3.6 Canopy density:
Canopy density was assessed through the fruiting zone using the Point Quadrat method (Smart and Robinson 1991) before leaf plucking. The percentage of gaps, leaf layer number, the percentage of interior leaves and interior clusters were taken at 10 cm intervals at 20 cm above the fruiting wire. A bay for measurement was selected randomly in each row from the trial site.

3.4.3.7 Leaf chlorophyll measurements:
The greenness or chlorophyll contents of the basal leaf opposite to the cluster were assessed using a Minolta-502 SPAD meter (Osaka, Japan). Readings of SPAD values were taken beginning at the season (1st November 2006), at veraison (17th-18th February 2007), pre harvest (27th March 2007) and post harvest (3rd and 5th April 2007). Two leaves per vine were recorded from the trial site, covering both eastern and western side of the canopy and four readings per leaf were averaged to get the leaf’s recorded value.

3.4.3.8 Nutritional status:
Petiole tissue for nutrient analysis was collected when flowering was completed (6th December 2006). Ten petioles per row from the trial site were sampled from leaves opposite the basal cluster in exposed shoots and the bulk sample of Control and Shell treatment tissues sent to R. J. Hill Laboratories Ltd (Hamilton, New Zealand) for a basic plant analysis. The elements analysed were N, P, K, S, Ca, Mg, Na, Fe, Mn, Zn, Cu and B.

3.4.3.9 Pruning evaluation:
Pruning data such as pruning weight and internode lengths were recorded from the middle portion of the slope in both treatments in July 2006 because greater variability was observed on the upper and lower part of the slope. The internode data were gathered
from whole shoots, measuring nodes positions 1, 2, 3 and 7, 8, 9. Node numbers per vine left at last pruning were recorded in the middle of November 2006. Pruning weight was also measured in July 2007 in all bays of the monitored experiment area. The internode data were also assessed in 18 whole shoots from each treatment.

3.4.4 Canopy environment

3.4.4.1 Canopy temperature:
Ambient air temperatures in the fruiting zone were recorded from 1st October 2006 to 30th June 2007 using Tiny Tag® logging sensors (Energy Engineering Ltd., Auckland, New Zealand) placed within a Stevenson-type screen in each treatment. Averages were calculated from temperatures recorded every 30 minutes.

3.4.4.2 Light measurements:
Ultraviolet radiation (UV-A and UV-B) and photosynthetically active radiation (PAR) were recorded after flowering (December 2006) and during grape ripening (March 2007). Measurements were taken from the 3rd bay in each treatment. The PAR sensor (Model Quantum, Lambda Instrument Corp., Massachusetts, USA) and, UV-A and UV-B sensors (Model Delta-T Devices, Cambridge, England) were located at fruiting level height to record incident PAR and UV-A light and reflected UV-B every 1 minute on 14th December 2006 and 7th March 2007. After one hour, sensors were rotated to record reflected PAR and UV-A and incident UV-B radiation. Sunny and cloudy conditions were considered on measurements.

3.4.5 Soil environment

3.4.5.1 Soil temperature:
Soil temperature monitoring at 10 cm depth from the ground surface (by Tiny Tag® loggers) were assessed from 1st November 2006 to 31st July 2007. Three probes were placed within each treatment, located at bay 4, rows 70, 72 and 74 for control, and rows 62, 64 and 66 for shell. Loggers recorded temperature every 30 minutes.

3.4.5.2 Weed assessment:
Weed development was studied in November during a peak of growth describing numbers and types. One bay per row was selected and weeds were counted and the
diameter of each was recorded using a tape measure. The coverage was estimated calculating the total leaf area (cm²) related to the bay area. Leaves were assumed to be circular.

3.4.6 Berry ripening

3.4.6.1 Veraison monitoring:
The progression of colour change through veraison was recorded on all vines of both treatments from the experiment area (90 vines per treatment). A visual assessment counting the number of berries coloured into the one cluster per vine was taken from 19th February to 5th of March every two days according to the Table 3-1. The monitoring was done always on the same cluster.

Crop estimation was completed during veraison. On 21st and 22nd of February two clusters per row were taken from the trial site and analysed according to cluster weight, wing and rachis weights and berry number. In addition, berries from each bunch were classified through three sizes small, medium and large (the latter defined as having a weight higher than one gram), and weights were determined using a Mettler Toledo scale (Model Viper SW3, New York, USA). Same berries were also classified according to four colours (green, pink, red and blue) completing an evaluation of ripening variability at veraison.

3.4.6.2 Maturity monitoring:
Grapes were analysed according to the method described by Iland et al. (2000). Several variables were recorded such as °Brix, titratable acidity and pH taking 45 single berries per row weekly from the 5th of March until harvest date. Berries were collected from the upper, middle and lower part of the cluster, considering the inside and outside surface. Skins and seeds from these samples were stored at -20°C for potential further analysis. At the same time, 30 single berries per row were frozen for potential aroma analyses later.

Peduncle lignification progression was monitored on all vines of the trial site at veraison and harvest stage (9th March and 26th March, respectively), according to a ranking which considered three levels of colour: full dark, moderate and green. One cluster per vine was considered for this measurement.
3.4.7 Harvest and winemaking

3.4.7.1 Harvest

The grapes for microvinification were hand harvested on 27th March 2007 from the experiment area and kept in cold storage overnight at 10°C. Table 3-2 shows where the fruit were harvested, mainly from bays three, four and five in each treatment area. Forty vines were harvested in the Control area and 69 from the Shells area in order to obtain enough fruit for the microvinifications.

Table 3-2. Map of trial showing location of vines harvested to obtain the fruit used for the 2007 microvinifications.

<table>
<thead>
<tr>
<th>Bay Number</th>
<th>Row Vine #</th>
<th>75 Cnt</th>
<th>74 Cnt</th>
<th>73 Cnt</th>
<th>72 Cnt</th>
<th>71 Cnt</th>
<th>70 Cnt</th>
<th>67 Shell</th>
<th>66 Shell</th>
<th>65 Shell</th>
<th>64 Shell</th>
<th>63 Shell</th>
<th>62 Shell</th>
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</tbody>
</table>

Fruit were harvested vine by vine and the fruit weight per bay recorded before the fruit was put into 40 kg baskets. The grapes for commercial wine were harvested on 27th March from the shell area and 31st March, from the control area.

3.4.7.2 Microvinification

The grapes from each treatment were processed through a destemmer-crusher (model Diemme, Lugo, Italy) on 28th March. °Brix, titratable acidity and pH were recorded and the juice separated into three 23 L food grade plastic buckets. Each bucket contained about 15 kg of crushed must and these were randomly allocated to replicates, three per treatment. Analyses such as °Brix, pH, titratable acidity and temperature were taken on 29th of March from the 6 musts, which were put in a warm room until their temperature reached 18°C (30th March).
The musts were inoculated with 200 ppm RC 212 yeast Bourgorouge Selection BIVB *Saccharomyces cerevisiae* (20 g/hl) at that time. Temperatures, °Brix and pH were monitored and recorded until fermentation was completed with 2 g/L of residual sugar. Caps were punched down by hand twice daily. An addition of DAP in one of the control containers was necessary due to H₂S production in mid-ferment (one 0.1 g/L dose). Both treatments’ wines took four days to complete fermentation, with temperatures peaking at 24°C halfway through. Completion of fermentation was tested through hydrometer readings and Bayer Clinitest® tablets.

On April 10 the contents of each fermentation container were pressed into an 11.3 L glass carboy (one for each replicate) by a manual vertical basket press. After pressing, wines were stored for one day to allow lees to settle and the following day (12th April) each carboy was racked and inoculated with malolactic culture (Ch 35 freeze-dried, at a 0.16 g/L rate) and left in the 20°C room. The headspace in each carboy was purged with CO₂ twice a week to protect wine against oxidation.

All wines were monitored by paper chromatography weekly until that they were considered to have finished malolactic fermentation (Illand *et al.*, 2000). After that, 50 ppm of SO₂ was added to all carboys on 27th of April and racked to smaller carboys.

The wines were then transferred out of the warm room and free sulphur was maintained at around 25 ppm until the wine was bottled in July. Alcohol, pH, titratable acidity and free SO₂ values were taken from each replicate and wines were put through a 1 micron cartridge filter prior to bottling in 750 cc bottle size.

### 3.4.7.3 Commercial vinification

The fruit was hand-harvested, then kept in a cool store overnight at 10°C and destemmed using crusher-destemmer (model Diemme, Lugo, Italy) the following day. A quantity of 50 ppm of SO₂ was added in both treatments and after a day soaking, musts were analysed for Brix, titratable acidity and pH.

Tartaric acid adjustment was made to the control treatment to reduce pH and increase acidity (0.75 kg/t). Pump over was practiced on the first day after destemming, and °Brix, temperature and pH were recorded every morning after hand plunging. Fermentation was
carried on with wild yeast at temperatures between 20 and 30°C, and wines were tested for dryness by Bayer Clinitest® tablets after approximately 9 days.

After 8 days macerating post fermentation, wines were pressed off using a press (model Diemme spa AR50Ns-A, Lugo, Italy) on a 1.5 hour maximum 1 bar cycle, mixing free-run and press fractions. After pressing, HE Grand Cru enzyme was added in both treatments (at 3g/L rate) and wines were put into barrel in May. Malo-fermentation went through natural bacterias, keeping wines at 20°C. It is expected that these wines will be blended and bottled in January 2008.

3.4.8 Phenolics composition

3.4.8.1 Wine Spectrophotometric Analysis
In November 2006, one bottle of each microvinification wine replicate (vintage 2006) was tested spectrophotometrically by the method of Iland et al. (2000) at Lincoln University by student Scott Hannan under the guidance of Dr Glen Creasy. Parameters included pH, wine colour density, wine colour hue, degree of red pigments colouration (expressed as %), SO₂ resistant pigments estimation, total red pigments and total phenolics which were measured on a Unicam UV4-100 spectrophotometer controlled by Vision software v3.10 (Unicam).

3.4.8.2 HPLC Analysis
An analysis of phenolic composition in wines and leaves was measured through High Performance Liquid Chromatography - Diode Array Detector (HPLC-DAD) as described by Keller et al. (2000). Grape skins had been analysed by Crawford (2006).

Increases of UV radiation and light should stimulate accumulation of leaf flavonols (Keller and Torrez-Martinez, 2004). For leaf extraction, ten leaf blade samples per row (6 rows each treatment) were collected prior to and post veraison and kept frozen at -20°C. Leaves were cut into small pieces and approximately 1g weighed into a 50 ml beaker and homogenised with 10mL cold (-20°C) acetone using an Ultra-Turrax Homogeniser. Samples were transferred to a screw-cap 50 mL centrifuge tubes and put on a rotating platform to mix for at least 30 minutes. After that, tubes were centrifuged at 3000 rpm for 10 minutes in a Megafuge 1.0 Heraeus (Thermo Fisher Scientific Inc., Waltham, USA). The supernatant was decanted and saved in another labelled 50 mL centrifuge tube, and
the pellet was washed once with 10 mL cold acetone again, and once with 10 mL cold acetone:MeOH (1:1 v/v), as before. The combined supernatants were centrifuged and kept at -20°C overnight. The following day, supernatant was decanted in another tube and the solvent was evaporated through nitrogen gas, keeping the bottoms of tubes in 35°C water bath. After that, sample volumes were transferred to small vials bringing them to complete dryness.

Sample extracts were redissolved prior to injection into the HPLC by adding 2 mL methanol to each vial, and then ultrasonication to dissolve the extract. A 0.5 mL aliquot of the solution were transferred to microfuge tubes and diluted with 0.5 ml of methanol. After 10 minutes centrifuging at 10,000 rpm, extracts were filtered using syringes and Teflon filters of 0.45 µm (LabServ Filtration, Biolab, New Zealand) into the autosampler vials. At the same time, three replicates of microvin as well as commercial wine samples from vintage 2006-2007 were also filtered prior to direct injection.

The HPLC equipment consisted in a model Waters 600-MS system controller, Waters™ 717 plus autosampler and Waters™ 996 photodiode array detector. A computer workstation with Millennium software v2.10 was used for chromatographic analysis. The column was a Phenomenex (Auckland, New Zealand) Luna 5µ C18 (2) 100A 250 mm length by 4.6mm ID. Run conditions were as shown in Table 3-3 (Keller et al., 2000). Solvent A was 2.5% v/v acetic acid/water and solvent B was 100% acetonitrile. Peak areas were calculated from chromatograms generated at 354nm for flavonols and 280nm for phenolics. Quantitative standards were used for phenolics compounds.

### Table 3-3. Run conditions for phenolic analysis. Solvent flow is 1.0ml per minute. Equilibration time between injections was five minutes.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Start A:B (%)</th>
<th>End A:B (%)</th>
<th>Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-40</td>
<td>100:0</td>
<td>70:30</td>
<td>Linear</td>
</tr>
<tr>
<td>40-50</td>
<td>70:30</td>
<td>10:90</td>
<td>Linear</td>
</tr>
<tr>
<td>50-55</td>
<td>10:90</td>
<td>--</td>
<td>Isocratic</td>
</tr>
<tr>
<td>55-60</td>
<td>10:90</td>
<td>100:0</td>
<td>Linear</td>
</tr>
</tbody>
</table>

Concentrations of flavonols (rutin and quercetin), flavan-3-ols (catechin and epicatechin) and p-coumaric acid were estimated at 280nm using standard curves ($r^2>0.99$) established with quercetin, catechin and p-coumaric acid standards, respectively. However, concentrations of gallic acid, caffeic acid and resveratrol were derived from
the absorbance at 280nm using the linear relationships between pure phenolic compounds and absorbance (Kolb et al., 2001).

### 3.4.9 Volatile aroma compounds analysis

A qualitative identification of volatile aroma compounds by using Solid Phase Micro-Extraction (SPME) Gas Chromatography-Mass Spectrometry (GC-MS) was performed at Lincoln University by student Tom Simpson under the guidance of Dr Rob Sherlock. The chromatograms obtained from one bottle of each microvin wine treatment (vintage 2007) were analysed using Shimadzu GC-MS Solutions Postrun analysis software. This software was used to search the spectral libraries for the possible compound that matched the mass spectra produced from each peak of the chromatograms. After that, peaks associated with compounds derived from the SPME process were discarded from the spreadsheet which included retention time, peak heights and areas. Retention time indices were compared to clarify individual compounds when there was ambiguity to identify them (Simpson, 2007).

### 3.4.10 Sensory Evaluation

The microvin wines from 2005 vintage and commercial wines from 2004 were taken to the Southern Pinot noir Workshop in January 2006 for evaluation by the delegates, who were winemakers from the New Zealand industry. Wines were scored according to the perception of bitterness, colour density, hue, total phenolics, ripe fruit, phenolic ripeness, palate texture and overall quality. Delegates marked their preferences on a scale (Appendix A) using as reference the Mouthfeel wheel (Gawel et al., 2000). The responses were quantified by measuring the distance from the origin of the line to each wine's mark, made by the panellists in the tasting sheet.
CHAPTER IV
RESULTS AND DISCUSSION

4.1 Grape quality

4.1.1 Cluster composition and yield components
Grapevine cluster make-up is an important consideration for both yield prediction and winemaking. Elements that determine berry quality such as °Brix, anthocyanins and phenolic compounds are influenced by sunlight intensity and its effect on the fruiting zone through photosynthesis and solar heating.

Figure 4-1. Yield components of 10 whole-basal clusters collected at harvest 2006 from the mid-slope region and stored at -20°C. Bars indicate standard errors of treatment means (n=10). A wing defined as group of berries arising from first branch on the rachis’ main axis and berries on it were not in these measurements.

Figure 4-1 shows that significant differences were found in the 2006 cluster yield components such as whole cluster weight and weight of cluster without its wing, with the values being higher in the Shell treatment (134 and 108, respectively). This difference was not due to rachis weight, but appeared to be due to slightly higher average berry weight (Figure 4-2) and somewhat greater berry number shown in Figure 4-1 (which refers to the number of berries in the cluster, not considering the wing), though both parameters are not statistically different between treatments. Average berry weight was
calculated in this experiment as the ratio of cluster weight (discounting the rachis weight) over berry number. However, heavier berries could be confirmed when bigger berries were found in shell clusters in a greater population of them analysed one by one (see section 4.1.2). Unfortunately it was impractical to survey more berries to look at all of them.

Although the percentage of fruit set was similar between treatments, the total numbers of berries were greater than control during season 2006/2007 (see section 4.2.6). However, fruit set was considered poorer in shell clusters because they showed seedless rather than seeded berries, being especially greater those seedless berries smaller than 3 mm.

During 2006/2007 season, bunches were heavier in the control treatment at about 90% of veraison completed with values being higher for whole cluster weights as well as for clusters without wings, although the variability of the sampling was also high, expressed by the standard deviations (see section 4.5.3). The total number of berries was greater in the shell treatment clusters, which may be related to better environmental conditions at flowering stage, resulting in a better percentage fruit set. However, fruit set was not improved in shell area based in lower numbers of seeded berries obtained. This result did not coincide with greater fruit set produced using clear plastic mulch (Creasy et al.,
and no significant effects on berry weight found using aluminised mulch (Coventry et al., 2005).

Regarding fruit composition (Figure 4-3), there were no measurable differences in °Brix, pH and titratable acidity (TA) during season 2005/2006, these results being similar those of Vanden Heuvel and Neto (2006) using a synthetic aluminised reflective mulch and crushed quahog shells (bivalve mollusc), and Extenday® mulch (Vanden Heuvel et al., 2007). Vines mulched with a white geotextile have had few effects on ripening time and fruit composition at harvest (Hostetler et al., 2006). However, it has been reported that berries have had slightly greater sugar content when the ground under vines was covered with a sheet of aluminium reflecting light (Spring, 2004; Coventry et al., 2005). A similar increase of soluble solids content has been found in Cabernet Sauvignon mulched with white foil (Todic et al., 2007) and other reflective foils developed for solarisation (Sauvage et al., 1998; 1996; Igounet et al., 1995). Yokotsuka et al. (1999) also increased berry °Brix using soils modified with oyster shells.

Figure 4-3. Fruit composition of whole-cluster samples collected at harvest 2006 from the mid-slope region and stored at -20°C prior to processing. Bars indicate standard errors of treatment means (n=10).
Regarding phenolic compounds, no significant differences were found between the treatments' extracted anthocyanins or total phenols from whole cluster samples using spectrophotometric methods (Figure 4-4). A similar lack of differences has been found in another recent study in Merlot and Pinot noir using white woven reflective matting called Extenday® (Vanden Heuvel et al., 2007). No effects of reflective mulches on anthocyanins and phenolics compounds under increases of light were also reported as well by Merwin et al. (2005). At the same time, other studies have shown no effect of exposure to full light on berry anthocyanin (Hannah et al., 2004; Price et al., 1995), though these last authors found increased total phenolic levels in the sun-exposed skins.

Figure 4-4. Spectrophotometric evaluation of phenolic extracts of whole cluster samples collected at harvest 2006 from mid-slope region. Berries were processed according to Iland et al. (2000). Bars indicate standard errors of treatment means (n=10).

Clearly, there was not effect of the reflective mulch on anthocyanins and phenolics compounds in this trial, contrary to positive effects on total anthocyanin concentration that have been found using a natural mulch of crushed quahog shells (Vanden Heuvel and Neto, 2006), which increased the concentration of red pigments by approximately 13%. A significant increase of total anthocyanin content in berry skin correlated to higher amount of PAR was also reported using white foil mulch, whereas total phenolics were not affected (Todic et al., 2007). Using aluminised
polyethylene sheeting, Conventry et al. (2005) increased total anthocyanins and phenolics in Cabernet franc, especially flavonol levels. In addition, grapes grown in soils modified with oyster shells also obtained higher amounts of total phenols and anthocyanins (Yokotsuka et al., 1999). Clearly, it seems to be that reflected light has affected grape phenolic contents, although this result could not be confirmed on this trial. However, there is a lack of results to discuss about the current finding of no effect on anthocyanins and phenolics content. The effect of reflective mulches on fruit composition would likely be dependent on weather conditions during the growing season, especially in cool climates (Vanden Heuvel et al., 2007). Robin et al. (2000) determined that red reflective mulch improved berry quality, increasing the relative amounts of blue anthocyanin pigments compared to red ones.

4.1.2 Berry variability within the cluster

Results from a sample of 1700-plus berries showed significant differences between treatments for berry weight, malate concentration and anthocyanins per berry, being all of them greater in shell berries. However, anthocyanin compounds per unit skin area was slightly lower in Shell treatment, indicating that berry weight was the reason for the greater anthocyanin amount in the Shell berries, due to a higher skin surface area (Table 4-1).

Table 4-1. Analyses of frozen fruit samples from 2005/2006 season - individual berry measurements. A p-value of less than 0.05 is regarded as being statistically significant.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Shells</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berry wt (g)</td>
<td>0.97</td>
<td>1.10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>°Brix</td>
<td>24.2</td>
<td>24.3</td>
<td>0.645</td>
</tr>
<tr>
<td>Malate (g/L)</td>
<td>0.34</td>
<td>0.40</td>
<td>0.042</td>
</tr>
<tr>
<td>Anthocyanins (mg/berry)</td>
<td>1.73</td>
<td>1.87</td>
<td>0.051</td>
</tr>
<tr>
<td>Anthocyanins (mg/cm2)</td>
<td>0.62</td>
<td>0.60</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Additional analysis of the data showed that berries in the Shell treatment had a positive shift in the population towards heavier berries than those in the Control treatment (Figure 4-5). This was also shown in the range of weights for each treatment, with values from 0.05g to 2.28g in the Control, and slightly higher, from 0.08g to 2.42g, in the Shells treatment.
Despite the fact that there were no significant differences in °Brix values between treatments, the shell mulch was successful in reducing the variability within clusters due to there being fewer low-value berries and fewer high-value berries contributing to the total sugar (Figure 4-6).

The absolute range in °Brix within the treatments was 13.4 to 41.5 in Control and 12.0 to 39.4 in the Shells. In this case, which is slightly different than was the case for berry weight, the extreme values were lower in Shells versus Control, but a slightly higher proportion of berries were riper, leading to a similar overall °Brix level, but somewhat reduced variation around the mean value (Figure 4-6). °Brix values were negatively correlated to fruit weight at harvest in both treatments, being greatest in small berries, which would suggest a higher rate of ripening in seedless berries (assumed to be those under 0.50g weight after analysing all of data) (see Appendix B).
In addition, the contribution of each class of berry weight to the total sugar available in the must was calculated for each treatment, showing that seedless berries, though having high sugar concentration (28°Brix), contributed only 3.5% of the total sugar in control and only 1.2% in shell grapes examined (Figure 4-7). On the other hand, large berries contributed 1.1% of the sugar in control and 6.1% in shells.

However, while seedless berries made up 10.7% of the anthocyanins (expressed in mg) for control and 5.6% for shell must, big berries contributed 4.4% and 19.6% of the total pigments, respectively (Figure 4-8).
Smallest and largest berries combined contribute 1/5 of the grape colour in shell must, being greater than control and demonstrating the relatively greater impact they have on potential wine colour. Overall, the largest contribution of sugar and anthocyanins came from berries with weights between 0.50 and 0.99 g (Figures 4-7 and 4-8), but the impact of the smallest and largest berries cannot be discounted.

This study has shown that there was considerable heterogeneity between different grapes collected from what would be considered to be a homogeneous area of vineyard. Some earlier studies have described large heterogeneity of developmental stages and sizes between berries in a cluster (May, 2000). In comparing fruit from two different areas (shell and control) in the vineyard, where shell fruit is under a higher regime of light than control, further differences in fruit composition and yield components were found. The differences found in berry weight and malate concentration could be related with the light and temperature conditions promoted by reflective mulch (Creasy et al., 2006). However, despite that °Brix levels were similar between treatments and anthocyanin amounts were a little bit lower in shell grapes, the variability in the fruit appeared to be decreased in the shell treatment. The largest as well as smallest berries had a significantly greater impact on potential wine colour in the shell compared to the control treatment. This response suggests a role of environmental factors over berry growth and metabolism processes, and that variability within the clusters can be altered through viticultural management.

The effects of light and temperature on berries are highly interactive because the temperature in the canopy is determined by the absorbed radiation and convective heat (Smart and Sinclair, 1976). The first growth stage, which may last around 60 days after
flowering (Possner and Kliewer, 1985) and where growth is by cell division and cell expansion, would be very sensitive to temperature (Ollat et al., 2002). In fact, berry growth is also slower when clusters grow without light during the initial developmental stages (Dokoozlian and Kliewer, 1996). However, measurement showed average hourly temperature to be cooler in the shell area compared to the control during the period of 60 days after flowering, with values being 18.2°C and 18.5°C, respectively (sed=0.21 in both cases) as it can be also seen in Figure 4.3-8 from 15th December to 15th February.

Additionally, it has been confirmed that delayed winter pruning implies an increase in the success of fertilisation of ovules, enhancing the quantity of seeded berries, and thus leading to greater berry development, although does not mean necessarily that warm temperatures at budbreak improve the ability of flowers to develop into fruit (Friend, 2005). Average hourly temperatures were quite similar between treatments from budbreak to flowering during 2005/2006 season, from around 30th September to 15th December (15.2°C for control and 15.1°C for shell, sed=0.2 in both cases), so this would not explain the bigger berries obtained in shell area during this season. However, the effect of greater growing degree hours recorded in shell area could have an impact on fruit set.

Similar results were found the previous season which recorded a warmer canopy above shell treatment by 1°C for 7.1 hours per day pre-veraison and 5.3 hours per day post veraison (Creasy et al., 2006). At the same time, it has been described that under controlled conditions berry growth increases as cluster exposure to sunlight increase, but in the vineyard, berry size also depends on the position in the canopy (Bergqvist et al., 2001).

°Brix values were similar between treatments, but the amount the sugar contained in each berry depended on the size, establishing a negative correlation where higher °Brix was obtained at smaller weights. Similar relationships have been found (Trought and Tannock, 1996), even depending on the position within the cluster. In fact, Tarter and Keuter (2005) suggested that the total °Brix of a cluster is poorly represented by berries sampled from its distal end.

It was expected to obtain lower malate concentration in Shell area due to higher light and heat into the berries. However, malate concentration was higher in the berries from the
Shell treatment. This may be due to the slightly lower temperatures in the Shells treatment fruiting zone recorded during the ripening at season 2006, even up to 0.4°C cooler than control in some instances (according to the Figure 4.3-9, section 4.3.2). At the same time, the temperature also was kept lower in the shell area just prior to veraison, when malic acid reached to the highest value during the first berry growth stage. Previous reports indicate that there is a negative correlation between temperature and malic acid due to the effect of temperature on the balance between malic acid synthesis and catabolism during ripening (Conde et al., 2007). Malic acid degradation is accelerated with high temperature because malic enzyme activity increases between 10 and 46°C (Ribéreau-Gayon et al., 2006a).

Overall, anthocyanin concentration was quite similar between treatments, although slightly lower in shell berries. However, small as well as larger berries contributed to potential wine colour. A photochemical effect of the solar irradiation on berry metabolism could be expected due to that photoreceptors could activate some enzymes of the flavonoid pathway (Pereira et al., 2006). It would seem to be that the response to radiation depends on its intensity and it is not necessarily a limiting factor for anthocyanin synthesis, though phenolic metabolism can be controlled by light quality (Bureau et al., 2000). Keller and Hrazdina (1998) showed that anthocyanin concentration in berries were similarly high at 20% and 100% sunlight interception.

On the other hand, the effect of the temperature is related to the activity of enzymes involved in the biosynthetic pathway due to optimum temperature for activity being between 17 and 26°C (Pirie, 1977, cited by Haselgrove et al., 2000). In fact, temperatures near to 17°C were reached in both shell and control treatments during the ripening period. However, it would seem that there is an effect of light which could be related to some increase of temperature.

4.2 Grapevine growth

4.2.1 Budbreak evaluation:
The progression of budbreak was monitored according to visual observations by Neudorf Vineyard staff. The information was collected during three dates (22nd and 29th September, and 9th October 2006) with no differences found in both treatments. According to these observations, budburst seemed to begin around the same date (22nd
September) in both treatments, showing green leaf tips in vines located in the shell area and a few first leaves in some bays of the control area. After one week, third leaves were seen more frequent in the control area, even with some inflorescences visible. Around 9th October 2006 it seems to be that bud burst was completed in both treatments most inflorescences visible and strong shoot growth evident.

Similar results were seen for the 2004/2005 season and although more detailed data were recorded about budburst during the 2005/2006 season, no differences were observed (Crawford, 2006). However, according to the information collected during several seasons (2004/2005, 2005/2006 and 2006/2007) it has been possible to see that budburst was completed later each year, being 21st September, 26th September and 9th October, respectively. It would seem that budburst date was not affected by shell mulch, despite lower soil temperatures and increases in light during this stage.

The length of the period between budbreak and flowering depends on the weather conditions. In September 2006, weather was warmer than average, with a mean air temperature of 11.7ºC, 207.3 hours of sunshine and low rainfall (78.8mm). A west to south west wind dominance was recorded on 21 days that month. October was slightly sunnier (with 222.9 hours of sunshine and mean temperature of 12.5ºC) and wetter than September, but with less rainfall than the average for this month (73.6mm on 12 rain days). Overall, weather conditions promoting warmer temperatures could favour budbreak. Accumulated temperature expressed as degree-days has the most effect, with 350 degree-days over a minimum period of 20 days required before flowering will take place (May, 2004). Shell mulch did not appear to increase the amount of GDD in this trial (see section 4.3).

It has been cited that changes in root temperatures have affected the length of the budbreak period as well: while moderate temperatures were reported to be necessary to budbreak and to bloom (25 to 30ºC) with lower as well as higher temperatures delaying these stages in a study using rooted vines planted in small containers kept in water baths (Kliewer, 1975), temperatures within the range 11 to 30ºC did not affect the duration of budbreak in a study using rooted cuttings (Woodham and Alexander, 1966). On Shell trial, soil hourly temperatures between bud-break and flowering in the 2005/2006 season were evidently lower in the shell area compared to the control, with values of 16.3 and 18.4ºC, respectively (sed 0.08 for shell; 0.12 for control). Although these results suggest
that shell mulch could be influencing the length of this period, taken to be between 20\textsuperscript{th} September and 15\textsuperscript{th} December, budbreak evaluation has not shown evidence of any effect (see section 4.4.1).

4.2.2 Shoot growth measurements:

The number of nodes laid at pruning in 2006, including spurs, was recorded. At the same time, the number of shoots and flower clusters per vines were counted previous to as well as after shoot thinning. The average number of nodes per vine laid at pruning was the same in both areas (about 26 nodes per plant), and there were no statistically significant differences between treatments in the number of shoots per vine grown during 2006/2007 season when it was recorded prior to and after shoot thinning (Table 4-2), demonstrating that the criteria to assess shoot thinning was the same in both areas. Shoot number recorded in both areas are in agreement with values between 10 and 15 shoots per metre row suggested to optimise the canopy light microclimate (Reynolds et al., 2005). In fact, Point Quadrat assessment showed similar trend (see section 4.2.7).

Table 4-2. Vine node number and shoot number in the trial area, November 2006. Confidence interval at 95%.

<table>
<thead>
<tr>
<th>Nodes per vine laid at last pruning</th>
<th>Shoot number per vine Pre shoot thinning</th>
<th>Post shoot thinning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Shoots</td>
<td>Control</td>
</tr>
<tr>
<td>Overall mean</td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>26.3</td>
<td>27.1</td>
</tr>
<tr>
<td>Std dev</td>
<td>3.16</td>
<td>4.18</td>
</tr>
<tr>
<td>N</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Conf int +-</td>
<td>0.65</td>
<td>0.86</td>
</tr>
</tbody>
</table>

In addition, contrary to expectation that greater light environment (more sun days) could influence initiation and differentiation in the previous season, resulting in a large number of inflorescences or clusters, shell treatment did not show an effect on the observed fruitfulness.

Table 4-3. Cluster per vine in the trial area, November 2006. Confidence interval at 95%.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Pre shoot thinning</th>
<th>Post shoot thinning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Shells</td>
<td>Control</td>
</tr>
<tr>
<td>Overall mean</td>
<td>46.3</td>
<td>28.2</td>
</tr>
<tr>
<td>Std dev</td>
<td>8.69</td>
<td>4.94</td>
</tr>
<tr>
<td>n</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Conf int +-</td>
<td>1.80</td>
<td>1.02</td>
</tr>
</tbody>
</table>
The number of clusters was approximately 46 in both treatments (Table 4-3) after shoot thinning, which would not have a direct influence on yield (see section 4.6).

Shoot growth and number of nodes per shoot were also analysed in November 2006 early in the season. It can be seen that neither shoot length nor number of nodes per shoot were different between treatments (Table 4-4). These results do not correlate to later measurements in the season such as longer internode lengths found in control when prunings were assessed in the season 2005/2006 and longer in the shell area during season 2006/2007.

Table 4-4. Vine shoot lengths and number of nodes per shoot in the trial area, 27th November, 2006. Confidence interval at 95%.

<table>
<thead>
<tr>
<th>Shoot length (cm)</th>
<th>Node number per shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Overall mean</td>
<td>89.1</td>
</tr>
<tr>
<td>Std dev</td>
<td>13.3</td>
</tr>
<tr>
<td>N</td>
<td>180</td>
</tr>
<tr>
<td>Conf int + -</td>
<td>1.95</td>
</tr>
</tbody>
</table>

These data indicate that shell treatment did not have an effect on shoot growth contrary to reports in a previous study in which high levels of UV reduced early season shoot growth (Keller and Torres-Martinez, 2004). Same study confirmed that UV stimulated lateral shoot growth, which was not found in this trial, where number and length of lateral shoots were quite similar between treatments (section 4.2.3). Although lower soil temperatures were recorded in the shell treatment (15.6°C versus 16.7°C, significant at p<0.05, between the 2nd and 10th November 2006), this did not appear to delay shoot growth as it was reported in potted vines (Zelleke and Kliewer, 1980). Warmer air conditions could favour growth (Keller et al., 2005), but canopy temperatures were not associated with changes in shoot growth the season 2006/2007 (Table 4-4). However, contrary to these results, earlier and greater shoot growth was measured in shells in November during 2004/2005 season (Crawford, 2006). More carbohydrate reserves may have been stored in perennial wood in the previous season (May, 1987), which could account for this difference.

4.2.3 Lateral production:

Lateral shoot counts and lengths were recorded beginning December before leaf plucking. By analysing means and confidence intervals in both treatments (Table 4-5) it
can be seen that no significant differences were found for the average number of lateral shoots per vine.

### Table 4-5. Number of lateral shoots per vine arising from the basal four node positions. Data recorded pre leaf plucking (n= 30; Confidence interval at 95%)

<table>
<thead>
<tr>
<th>Lateral shoot / vine</th>
<th>Control</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node position</td>
<td>Average</td>
<td>Conf + -</td>
</tr>
<tr>
<td>Node 1</td>
<td>2.3</td>
<td>0.62</td>
</tr>
<tr>
<td>Node 2</td>
<td>11.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Node 3</td>
<td>12.6</td>
<td>0.74</td>
</tr>
<tr>
<td>Node 4</td>
<td>12.8</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Lateral shoot length means at the same node position of main shoots were similar in both treatments (Table 4-6). Using lateral production as a dimension of vine vigour, it seems to be that shells are not affecting vine growth as it might be expected if soil moisture and light are increased by shells (Crawford, 2006). However, it is not certain why this difference was not found.

### Table 4-6. Length (expressed in cm) of lateral shoots arising from the basal four node positions of shoots taken previous leaf plucking from 30th November to 5th December 2006. Confidence interval at 95%.

<table>
<thead>
<tr>
<th>Average lateral shoot length (cm)</th>
<th>Control</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node position</td>
<td>Average</td>
<td>Conf + -</td>
</tr>
<tr>
<td>Node 1</td>
<td>0.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Node 2</td>
<td>5.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Node 3</td>
<td>8.5</td>
<td>1.50</td>
</tr>
<tr>
<td>Node 4</td>
<td>10.4</td>
<td>1.70</td>
</tr>
</tbody>
</table>

Vegetative growth expressed in variables such as shoot number, lateral growth, number of nodes per shoot, and later, canopy density were not affected by shells. Only shoot length was statistically lower in the shell area. It is possible that these values are rather related to some effect of lower soil temperatures (Zelleke and Kliewer, 1980) or increases of UV radiation (Keller and Torres-Martinez, 2004).

#### 4.2.4 Trunk circumferences:

Trunk circumferences were measured on two dates, November 2006 and September 2007, from 90 and 30 vines per treatment respectively, using a tape measure around the first internode above the graft union. This measurement has been used as an indicator of cumulative plant vigour (Strong and Azarenko, 2000; Heazlewood et al., 2006). No
significant differences were found between treatments at either date (Table 4-7). However, these data indicate an increase between the two measurement dates in shell area. It will be interesting to see if this increase remains over the next few years. By looking at the average rate of increase between two dates for each treatment from 30 vines, no significant differences were found between them, being 0.95 cm in control (sed=0.18), and 0.85 cm in shell area (sed=0.07), though there was lower variability in shell vines which was demonstrated by a lower standard deviation.

Table 4-7. Trunk circumference data (cm) collected in November 2006 and September 2007. Confidence interval at 95%.

<table>
<thead>
<tr>
<th></th>
<th>Nov-06</th>
<th>Sep-07</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Shells</td>
</tr>
<tr>
<td>Mean</td>
<td>10.04</td>
<td>10.11</td>
</tr>
<tr>
<td>Std dev</td>
<td>1.29</td>
<td>1.06</td>
</tr>
<tr>
<td>n</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>Conf int + -</td>
<td>0.27</td>
<td>0.22</td>
</tr>
</tbody>
</table>

4.2.5 Progression of flowering:

The progression of flowering was assessed between 25\textsuperscript{th} November and 13\textsuperscript{th} December during 2006/2007 season using a visual scoring system (Table 3-1, section 3.4.3).

Figure 4-9. Progression of flowering during 2006/2007 season. Error bars are standard errors of treatments means (n=90, Control; n=89, Shell). Confidence interval at 95%.
According to the ranking recorded (Figure 4-9), flowering was more advanced in the Shell treatment than Control for the whole period monitored, which was similar to the situation observed for the 2004/2005 season (Crawford, 2006). In looking at the trends, flowering could have finished slightly sooner than in the control, approximately five days in advance during December 2006. At the same time, this advancement was more evident beginning the period evaluated and from the 4th and 9th of December when there were significant differences in the score between treatments.

Air temperatures and rainfall were very changeable at bloom stage in the Nelson area. November had frequent fluctuations in temperature, but the mean temperature was 14.5°C and the total sunshine hours 236.8. Daytime and night temperatures were warmer compared to the long term average, the warmest day being the 23rd with 22.4°C recorded, when flowering was beginning. Rainfall was 96.6mm over 15 days, which was above average for this month. However, December was a cool and breezy month with high sunshine (262.5 hours) and light and sporadic rainfall (over 18 days with 44.4mm being lower than average). The mean air temperature was 14.8°C, being considerably cooler than average for this month.

Temperature has major effects on pollination and fertilisation just before and during anthesis, influencing directly the development of sexual parts of the flower and indirectly the vine growth, which impacts flower development and fruit set (Koblet, 1966; Ebadi et al., 1995a; May, 2004). In addition, it seems to be that temperature may have some effect on flower size. High variation in flower size exists within inflorescences (May, 1987), with flowers of different diameters opening according to the location in the inflorescence (Friend, 2005). This variation has been seen in flowers developing at 12°C as well as 28°C, although variation is lower at high temperature (Ezzili, 1993).

Temperatures between 18 and 20°C intensify cap fall (Winkler et al., 1974). However, low temperatures and rainfall disrupt (May, 1987) and increase the duration of cap-fall (Staudt, 1999), suggesting a delay of the flowering process. So increasing the speed of flowering could be more beneficial than later flowering to advance fruit set and at the same time the ripening and harvest date, especially considering that flowering occurs over a two to three week period under the New Zealand maritime climate, and this interval can be longer in cool and wet springs (Friend, 2005). In fact, flowering has been
related to budburst and harvest time (Barbeau et al., 1998) and its prediction is important to determine an early estimate of harvest date (May, 2004).

However, shells only promote warmer temperatures in the canopy for a longer period in a daytime only (see Figure 4-12), which could explain the slight advancement seen in shell bunches during the monitoring period, though this improvement does not represent an advance of the ripening period or harvest date. The shell mulch did not increase mean hourly temperatures or GDD during the whole season, which may be why the treatment had relatively little impact on related factors.

### 4.2.6 Fruitset

Prior to bloom, a number of inflorescences were enclosed in a net bag to retain all of the flower caps. The bags and contents were removed two weeks before full veraison and frozen at -20°C. Samples were analysed later, drying bunches, counting shed caps and classifying individual berries according to their sizes. The percent of fruit set was calculated as the quotient of the total number of berries over the total of caps (flowers) per inflorescence.

<table>
<thead>
<tr>
<th>Mean</th>
<th>Conf int + -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Number of caps</td>
<td>318</td>
</tr>
<tr>
<td>Number of berries</td>
<td>130</td>
</tr>
<tr>
<td>Fruit set (%)</td>
<td>42.6</td>
</tr>
</tbody>
</table>

Table 4-8 shows that the number of berries per bunch was statistically different between treatments, being greater in shell area. However, the percentage fruit set was statistically similar between shell and control, demonstrating that shells did not improve fruit set.

Types of berries were also analysed during fruit set assessment. Although the number of larger seedless berries (Seedless A) and seeded berries were similar between treatments, only smaller seedless berries (Seedless B) were different between treatments. It can be deduced from Table 4-9 that the proportion of flowers that developed into smaller seedless berries was greater in bunches coming from the shell area, accompanied by a
reduction in the proportion of flowers that formed seeded berries. This fall would be based in higher number of total berries recorded (Table 4-8).

Seedless berries were classified according to diameters A and B (4 to 5mm, and ≤3mm, respectively), due to browning during the drying process, which meant they could not be classified by colour. However, as few green berries were seen at veraison assessment and harvest time, it was assumed that all of berries smaller than 3mm were coloured and in consequence classified as seedless berries. Hence, fruit set was affected negatively by reflective mulch due to lower numbers of seeded berries over the total floret number, setting more small berries.

Table 4-9. Number of different type of berries classified during fruit set assessment. Confidence interval at 95%.

<table>
<thead>
<tr>
<th>Type of berry</th>
<th>Diameter (mm)</th>
<th>Control</th>
<th>Shell</th>
<th>Control</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeded</td>
<td>≥6</td>
<td>45.5</td>
<td>38.3</td>
<td>5.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Seedless A</td>
<td>4-5</td>
<td>44.6</td>
<td>56.2</td>
<td>6.9</td>
<td>17.7</td>
</tr>
<tr>
<td>Seedless B</td>
<td>≤3</td>
<td>39.4</td>
<td>85.4</td>
<td>8.9</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Fruit set would be strongly influenced by supply of assimilates to the inflorescence during and after anthesis (Coombe, 1973), where sufficient leaf area provides assimilates for fruit development (Keller and Koblet, 1994). Zinc, Boron and Molybdenum play a large role in affecting fruit set (Robinson, 2005). Lower level of Molybdenum (Table 4-11) could be producing greater seedless berries or millerandage in shell area (Longbottom et al., 2005).

Fruit set was assessed classifying berries according to their sizes, identifying them such as seeded and seedless berries. Although it was not possible to describe berries as hen, chicken or shot berries (Friend et al., 2003) because berries were damaged by the drying process. Berries were categorized in a similar way as described by Heazlewood et al. (2006). Pinot noir produces seeded berries normally and is not considered as a stenospermocarpic or parthenocarpic variety (Friend et al., 2003). Despite this, fewer seeded berries were produced in shell treatment based on average seed number.

Flower abscission may be explained by hormonal balance perturbation, poor climatic conditions during flowering (Jackson, 1991) or some nutrient deficiency in the inflorescences (Gu et al., 1996). Some cultivars differ in their sensitivity to
environmental factors that can cause flower abscission (Lebon et al., 2004). Low temperatures may disrupt ovule development (Ebadi et al., 1995b) and reduce pollen viability (Koblet, 1966), pollen germination and pollen tube growth (Staudt, 1982), which could promote the formation of shot berries. In fact, Friend et al. (2003) confirmed that low temperature conditions during the earlier period of capfall may result in a greater proportion of smaller flower sizes setting fruit. Temperature seems to have an effect on fruit-set more so than light intensity. Low average temperatures (below 15°C) and high temperatures (above 35°C) affect fruit set negatively (Kliwer, 1977; 1975). In fact, fruit set was poorer in the shell area when temperatures recorded at flowering were around 15°C (Figure 4-13, section 4.3.2). This is in agreement with a study where Chardonnay vines exposed to low temperatures just before and during flowering suffer significant reduction in fruit set and increases of millerandage (Ebadi et al., 1995a).

Under controlled conditions, fruit set has not been modified by shading vines by 99% one week before full bloom (Deloire et al., 1995). However, slightly lower fruit set has been obtained by reducing light intensity (Ollat, 1992). The same trend was seen when vines were in full light or heavily shaded from 5 days before and 10 days after flowering (Nuno, 1993). Unfortunately, it is unclear if fruit set in the present trial was affected by lower photosynthesis or altered flower development. It should be noted that when inflorescences were bagged before and after anthesis, fruit set in the vines was not affected (May, 2000).

The effect of low light is combined with low temperatures in field conditions. Reducing light intensity decreased temperatures (15 and 10°C, for day and night respectively) and at the same time fruit set (Roubelakis and Kliwer, 1976). The same study showed that the highest light intensity produced a greater proportion of seedless berries (chickens). This could explain greater seedless berries number found in the shell area.

4.2.7 Canopy density:

The Point Quadrat method was used to assess the canopies, using one bay per row in the experimental area (6 bays per treatment). Parameters such as leaf layer number, percentage of internal leaves, internal clusters and gaps were calculated from the measurements. Canopy density showed no significant differences through the fruiting zone between the treatments; in fact, vines showed desirable values for most canopy
characteristics (Table 4-10). However, the percentage of interior leaves and clusters were slightly greater on the shell area, though not statistically different.

### Table 4-10. Point Quadrat assessments taken 5th December, 2006. Confidence interval at 95% and n=6.

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Std Dev</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cnt Shell</td>
<td>Cnt Shell</td>
<td>Cnt Shell</td>
</tr>
<tr>
<td>% gaps</td>
<td>7.3 9.7</td>
<td>4.5 6.5</td>
<td>3.6 5.2</td>
</tr>
<tr>
<td>Leaf layer number</td>
<td>1.9 2.0</td>
<td>0.3 0.4</td>
<td>0.2 0.3</td>
</tr>
<tr>
<td>% interior leaves</td>
<td>22.8 23.8</td>
<td>4.9 5.6</td>
<td>3.9 4.5</td>
</tr>
<tr>
<td>% interior clusters</td>
<td>34.3 37.1</td>
<td>6.9 19.7</td>
<td>5.6 15.8</td>
</tr>
</tbody>
</table>

Similar results were found in the 2004/2005 season, when the Point Quadrat assessment was done prior to leaf plucking in the same area (Crawford, 2006). Thus, it can be suggested that shells are not increasing vigour, which is desirable as this could lead to shading and lower fruitfulness and bud initiation, affecting quality and fruit composition negatively (Smart et al., 1991; Sommer et al., 2000; Cortell and Kennedy, 2006; Ristic et al., 2007). Conversely, improving sunlight exposure could be associated with more potential fruitfulness and increased bunch size (Archer and Strauss, 1989; Koblel, 1996; Sommer et al., 2000; Keller et al., 2005; Sánchez and Dokoozlian, 2005), affecting yields. However, it is not clear yet the quantity or length of sunlight exposure necessary to reach maximum levels of bud fruitfulness, or if there is a saturation level as a response to solar radiation (Sánchez and Dokoozlian, 2005). These same authors confirmed that the temperature optimum for bud fruitfulness is higher than for vegetative growth, and that shoot light microclimate rather than individual bud light interception is the most important factor determining bud fruitfulness.

### 4.2.8 Nutritional status:

Table 4-11 shows that nutrient values were different in both types of tissues tested: petioles and blades. More details can be seen in Appendix G. While N and S were higher in blade samples compared to petiole ones, the levels of Mg were higher in petiole than blade samples. Although no statistical data are available from these analyses, it is possible identify some trends. By comparing blade and petiole samples in both treatments, N concentration was higher in the shell treatment. The table also indicates that Ca levels were higher in the shell area compared to the control.
Table 4-11. Nutrient analyses of blade and petiole samples collected from opposite the basal cluster. Ten leaves with petioles were taken per row at the end of November 2006 (during flowering) and combined before analysis, as according to Hill Laboratories guidelines. No statistical data are available. Medium range is proposed as adequate values by the laboratory.

<table>
<thead>
<tr>
<th>Element</th>
<th>Unit</th>
<th>Shell Blades</th>
<th>Control Blades</th>
<th>Medium Range</th>
<th>Shell petioles</th>
<th>Control petioles</th>
<th>Medium Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>%</td>
<td>2.80</td>
<td>2.50</td>
<td>2.8-3.4</td>
<td>1.20</td>
<td>1.10</td>
<td>0.8-1.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>%</td>
<td>0.31</td>
<td>0.26</td>
<td>0.22-0.35</td>
<td>0.24</td>
<td>0.22</td>
<td>0.18-0.45</td>
</tr>
<tr>
<td>Potassium</td>
<td>%</td>
<td>0.80</td>
<td>0.80</td>
<td>1.1-1.5</td>
<td>0.80</td>
<td>0.90</td>
<td>1.7-2.7</td>
</tr>
<tr>
<td>Sulphur</td>
<td>%</td>
<td>0.40</td>
<td>0.35</td>
<td>0.30-0.50</td>
<td>0.18</td>
<td>0.18</td>
<td>0.13-0.25</td>
</tr>
<tr>
<td>Calcium</td>
<td>%</td>
<td>2.19</td>
<td>1.58</td>
<td>1.2-2.0</td>
<td>2.17</td>
<td>1.45</td>
<td>1.3-2.1</td>
</tr>
<tr>
<td>Magnesium</td>
<td>%</td>
<td>0.29</td>
<td>0.30</td>
<td>0.2-0.4</td>
<td>0.53</td>
<td>0.55</td>
<td>0.30-0.60</td>
</tr>
<tr>
<td>Sodium</td>
<td>%</td>
<td>0.02</td>
<td>0.02</td>
<td>0-0.1</td>
<td>0.03</td>
<td>0.02</td>
<td>0-0.15</td>
</tr>
<tr>
<td>Iron (mg/Kg)</td>
<td></td>
<td>74</td>
<td>81</td>
<td>40-150</td>
<td>27</td>
<td>26</td>
<td>20-50</td>
</tr>
<tr>
<td>Manganese (mg/Kg)</td>
<td></td>
<td>750</td>
<td>640</td>
<td>40-200</td>
<td>140</td>
<td>210</td>
<td>25-140</td>
</tr>
<tr>
<td>Zinc (mg/Kg)</td>
<td></td>
<td>860</td>
<td>610</td>
<td>25-80</td>
<td>120</td>
<td>140</td>
<td>25-60</td>
</tr>
<tr>
<td>Copper (mg/Kg)</td>
<td></td>
<td>8</td>
<td>26</td>
<td>6-12</td>
<td>11</td>
<td>14</td>
<td>5-20</td>
</tr>
<tr>
<td>Boron (mg/Kg)</td>
<td></td>
<td>126</td>
<td>112</td>
<td>28-45</td>
<td>57</td>
<td>44</td>
<td>28-40</td>
</tr>
<tr>
<td>Molybdenum (mg/Kg)</td>
<td></td>
<td>0.16</td>
<td>0.13</td>
<td>0.15-0.50</td>
<td>0.05</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Nitrate-N</td>
<td>(mg/Kg)</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>500-2000</td>
<td>1060</td>
<td>270</td>
<td>400-1600</td>
</tr>
</tbody>
</table>

It has been accepted that high concentrations of calcium contribute to delayed senescence (Chardonnet and Donèche, 1995). It is possible that the altered calcium levels could be related to the greener peduncles in the shell area (see section 4.5). Potassium levels were no different between treatments, but they were lower than the medium range. Reuter and Robinson (1986) suggested levels of 1.5% K in petioles at flowering time. Nonetheless, values for Mg were in the normal range, but no differences were found between treatments from blade and petiole samples. A correlation of SPAD values with levels of P, K and Mg has been found in other studies (Porro et al., 2001), being even stronger for N and chlorophyll due to the importance of N to photosynthesis. In fact, nitrate-N was much higher in shell petioles, with its value in the range suggested to improve fruit set (May, 2004).

Leaf blade and petiole analysed in 2006/2007 season could be related to soil analyses carried out in the 2004 and 2005 reported by Crawford (2006). High levels of calcium were found in soil under shells in both seasons before (Crawford, 2006). The same trend is still apparent in the shell area according to leaf and petiole analyses. High nitrogen in blades and petioles from Shell area could be correlated to high levels in soil. However, potassium and magnesium were not different between treatments when blades and petioles were analysed. Potassium was lower in soil collected from Shell area in 2004.
and 2005, and magnesium was higher in shell soil samples in 2005 and lower in 2004. More details can be seen in Appendix F.

4.2.9 Pruning evaluation:
Soil moisture and high levels of available nitrogen have resulted in increases of vine vigour (Smart, 1985). In effect, soil moisture has been higher in the shell area compared to the control area, so an increase of vigour could be expected (Crawford, 2006). Pruning weight is considered as a measurement of vigour (Rives, 2000). Therefore, pruning data were recorded for 2006 and 2007 seasons.

According to the Table 4-12, although statistical analysis were not possible for pruning weight in season 2006, it appears that shoots from the control treatment were less heavy than the shells. Pruning weights taken in July 2005 showed significant differences between treatments, with weights being higher in shells (Crawford, 2006). In both seasons samples were collected from the middle of the block to decrease the influence of the variability between upper and lower places. However, pruning weights were recorded in September 2007 from the experimental area (lower area of the upper block), indicating no significant differences between treatments. The changes between years could be explained through the variability existing in the block and by different conditions of rainfall, temperatures and crop load developed in each season.

Table 4-12. Pruning weights. No statistics are available for the pruning weight data in season 2005-6. Confidence intervals at 95% are 0.46 for control and 0.32 for shell in season 2006-7.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2006¹</th>
<th>2007²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.65</td>
<td>8.19</td>
</tr>
<tr>
<td>Shell</td>
<td>6.40</td>
<td>7.82</td>
</tr>
</tbody>
</table>

¹ Pruning data taken from the mid-portion of the slope by Neudorf Vineyard staff.  
² Pruning data taken from 18 bays (5 vines per bay) included in the trial site.

Fruit yield to pruning weight ratios, known as Ravaz Index (Ravaz, 1930), were calculated and vine capacity, which estimates the annual above-ground dry matter production, was estimated by the formula (yield*0.25) + (pruning weight*0.55), being the percentage of dry matter content of fruit and prunings, respectively (Winkler et al., 1974).
Both parameters, Ravaz Index and vine capacity, were statistically different between treatments, being higher in the control (Table 4-13). These results are in agreement with previous studies (Crawford, 2006), in which Ravaz Index was also lower in shells during the season 2004/2005 but contrary to higher values found in shell than control during 2003/2004. However, dry matter production was greater in control area in the 2006/2007 season, contrary to measurements recorded previously in 2004/2005 and 2003/2004, demonstrating that a great variability exists between seasons (Crawford, 2006).

Table 4-13. Ravaz Index and vine capacity for season 2006/2007. Confidence interval at 95%.

<table>
<thead>
<tr>
<th>Ravaz Index</th>
<th>Vine capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Mean</td>
<td>0.89</td>
</tr>
<tr>
<td>Std dev</td>
<td>0.22</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
</tr>
<tr>
<td>Conf int + -</td>
<td>0.15</td>
</tr>
</tbody>
</table>

If the Ravaz Index and vine capacity values are re-calculated, using predicted yield at veraison (including factor 1.3) and assuming that fruit had not fallen off the vine, the Ravaz index is increased (Control 2.27 and Shell 1.45) and vine capacity decreased (Control 1.83 and Shell 1.43). These values are closer between treatments to those seen previously. This hypothesis would explain that yield was lost in shells between crop estimation time and harvest due to shrivelling seen in shell grapes, specially affecting the large population of small berries. Similar findings were described by Crawford (2006) who reported a lot of berry shrivels during the 2004/2005 season which meant that final yields were lower than those estimated. However, it can be suggested that predicted yield may give a better indications about Ravaz index and vine capacity parameters.

Although the Ravaz Index values obtained in this trial were lower than those described in previous studies (between 5 and 10, Smart and Robinson, 1991; Vasconcelos and Castagnoli, 2000; and even in a range from 3 to 6 for Pinot noir grown in cool climates, Kliewer and Dokoozlian, 2005), well balanced vines in the shell area are achieving both fruit quality and consistent production by showing values up to 1kg pruning weight per meter of canopy length (data not shown). In fact, Kliewer et al. (2000) suggested values up to 1kg/m pruning per meter for Cabernet Sauvignon to produce wine without a loss in productivity due to excessive canopy shading, though previously values of 0.3 to 0.6kg had been proposed (Smart and Robinson, 1991). Crawford (2006) confirmed that these
low crop load: pruning weight ratio values are more characteristic of New Zealand conditions.

Additionally, it seems to be that lower vine capacity found in shell area after pruning assessment during season 2006/2007 is not related with shoot growth at the beginning of season. Bennett et al. (2005) reported that shoot growth slowed after 25 days post-budburst for defoliated vines (in previous season) in conjunction with reduced vine capacity, suggesting a close relationship between available CHO reserves beginning the season and vine productivity during the growing period. However, despite the higher pruning weights found in the shell area during season 2005/2006 and lower SPAD values measured in shell vines post-budburst during the spring in 2006, shoot growth in the shell area was similar to that of the control, being at the same time correlated to similar pruning weight at the end of the season 2006/2007.

The accumulation of carbohydrates in vines as reserves depends on the rate of photosynthesis and the partitioning that they have to shoot, root, fruit and storage (Howell, 2001). Although also there were no differences in lateral shoot growth and trunk circumferences between treatments, which would confirm no differences in terms of the accumulation of reserves, further data collection analysing CHO levels in trunks and roots at different phenological stages would be necessary to clarify this similar growth between treatments.

An increase of vigour was seen in past seasons (2005 and 2004) in which greater pruning weights were found in the shell area. Although an increase in internode length was not recorded, Crawford (2006) suggested that greater diameter of the shoots may reveal differences. However, cane diameter was not measured in the 2005/2006 season.

Table 4-14. Average internode length in two seasons from 20 shoots collected randomly post pruning. p-value<0.05 is considered to be statistically significant.

<table>
<thead>
<tr>
<th></th>
<th>Average internodes length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.2</td>
</tr>
<tr>
<td>Shell</td>
<td>6.9</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sed</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Table 4-14 shows that internode lengths were significantly different between treatments for 2005/2006 season, being shorter in the shell area than control. Interestingly, shoot growths were similar in both areas at the beginning the season 2006/2007 (refer Table 4-4). However, longer internode lengths in shell area were found at pruning in September 2007. So, it could be inferred that not only there is some effect of the reflective mulch on vine growth depending on the seasonal conditions, but also other variables such as increased water availability could be influencing shoot growth, though this parameter was not evaluated during 2006/2007 season. A higher pruning weight and speed of elongation of shoots would be expected under higher water content in soil (Rives, 2000) as it was described for shell area during 2004/2005 season (Crawford, 2006).

Table 4-15. Internode length versus position along the shoot in two seasons. \( p < 0.05 \) is considered to be statistically significant between treatments.

<table>
<thead>
<tr>
<th>Internode length (cm) at node position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>p-value</th>
<th>sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>0.61</td>
</tr>
<tr>
<td>Control</td>
<td>4.0</td>
<td>6.3</td>
<td>8.1</td>
<td>10.5</td>
<td>10.2</td>
<td>10.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shell</td>
<td>3.2</td>
<td>5.5</td>
<td>7.9</td>
<td>8.6</td>
<td>8.3</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.006</td>
<td>0.58</td>
</tr>
<tr>
<td>Control</td>
<td>3.5</td>
<td>5.6</td>
<td>7.5</td>
<td>7.9</td>
<td>7.9</td>
<td>7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shell</td>
<td>2.9</td>
<td>5.0</td>
<td>8.1</td>
<td>10.2</td>
<td>8.3</td>
<td>8.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Internode lengths measured on 20 shoots taken from the mid-portion of the slope.
² Internode lengths measured on 18 shoots taken randomly from trial site.

Table 4-15 shows data taken from pruning samples gathered from each treatment area during two seasons. Internode length was recorded from whole shoots, with internodes 1, 2, 3 and 7, 8, 9 being measured. Significant differences exist for internode position between treatments in season 2006, being much longer in the control compared to shell for all of node positions and even further out on the shoot these differences were more pronounced. However, internode length was also statistically different between treatments during 2007, but being longer in control for the first two internode position from the base and greater in shell for nodes situated further out on the shoot.

It is known that the Shell treatment increases the amount of UV radiation reflected into the canopy (Crawford, 2006; Section 4.3.3), and it could be that this is changing the formation of nodes on the shoots. Past research has shown that UV radiation alters photosynthesis and growth (Jansen et al., 1998). However, shell treatment had not a significant effect on the pruning weights in 2006/2007 season, contrary to that it
happened in past seasons when greater pruning weights were obtained under solarisation (Robin et al., 1997).

Seasonal weather differences could be influencing these results. It seems to be that season 2005/2006 was wetter than season 2006/2007, which could increase the water available in a clay soil for longer time, impacting the speed of elongation of the shoots and finally raising pruning weights as it was described by Rives (2000). However, if mean hourly temperatures recorded between 1st November and 31st March are compared between both seasons, clearly season 2005/2006 was warmer than 2006/2007 with values of 17.36°C and 17.12°C for control and shell in the previous season, and 16.6°C and 16.7°C, respectively, during the last season. These differences could vary vine growth in two different seasons. In fact, Howell (2001) has suggested that the application of vine balance concepts in cool climate regions has complications due to a strong annual fluctuation in weather conditions.

4.3 Canopy environment:

4.3.1 Leaf chlorophyll measurements:

Leaf greenness was measured at four different stages of growth: early season shoot development, veraison, pre-harvest and post harvest using a Minolta 502 SPAD meter (Soil Plant Analysis Device, Konica Minolta Sensing Inc., Osaka Japan). There is a correlation between the SPAD measurements not only with the level of photosynthesis occurring in vines, but also with the amount of carbohydrates which could potentially be produced (Candolfi-Vasconcelos et al., 1994).

Figure 4-10 shows that the shell treatment had higher readings than the control for all of stages measured except after bud break. This situation could be due to the increased UV radiation in the shell treatment, leading to decreased leaf greenness and, potentially, photosynthetic rates (Nunez-Olivera et al., 2006; Lafontaine et al., 2005; Schultz, 2000; Jansen et al., 1998; DeLucia et al., 1992) meaning a possible disadvantage early in the season. A similar condition was observed in grapevines at 2-5 leaf stage grown under solarisation, where practically there was as adaptation to the stress radiation (Robin et al., 1997). However, it was confirmed that UV under field conditions had no effect on leaf chlorophyll and gas exchange (Keller and Torres-Martinez, 2004) compared to ambient
UV reduced using diacetate film suspended above the plants, which transmitted around 2% UV-B and UV-A, and 93% PAR.

![SPAD measurement](image)

Figure 4-10. SPAD (leaf greenness) measurements in different stages during season 2006/2007. Larger values indicate higher levels of greenness. Error bars are shown at 95% confidence interval.

Figure 4-10 shows that the shell treatment had higher readings than the control for all of stages measured except after bud break. This situation could be due to the increased UV radiation in the shell treatment, leading to decreased leaf greenness and, potentially, photosynthetic rates (Nunez-Olivera et al., 2006; Lafontaine et al., 2005; Schultz, 2000; Jansen et al., 1998; DeLucia et al., 1992) meaning a possible disadvantage early in the season. A similar condition was observed in grapevines at 2-5 leaf stage grown under solarisation, where practically there was as adaptation to the stress radiation (Robin et al., 1997). However, it was confirmed that UV under field conditions had no effect on leaf chlorophyll and gas exchange (Keller and Torres-Martinez, 2004) compared to ambient UV reduced using diacetate film suspended above the plants, which transmitted around 2% UV-B and UV-A, and 93% PAR.

On the other hand, by veraison leaves on vines in the shell treatments were significantly greener than those in the control, and this relationship held through post harvest (time of the biggest difference between treatments, 6.9 units). These greater chlorophyll levels
found in fruiting zone leaves in this study agree with the improved photosynthesis status reached in low leaves of the canopy under solarisation (Robin et al., 1997). While canopies consist of leaves of different ages, which are exposed to different light intensities during the growing season (Hunter and Visser, 1988), the absorbance of PAR (400-700 nm) is highest in leaves fully matured but non-senescent at any time during the season, in contrast with young apical and old basal leaves (Schultz, 1996).

Leaf chlorophyll content has been positively (Cortell et al., 2007) and negatively (Cortell et al., 2005) correlated to vigour in Pinot noir. However, although shoot lengths, number of nodes per shoot and canopy vigour were not different between treatments during this season (2006/2007), more chlorophyll was found in leaves located in the shell area, even when SPAD values decreased due to the leaf senescence leading up to post harvest. Candolfi-Vasconcelos et al. (1994) also confirmed that older main leaves opposite to the clusters had lower chlorophyll content than lateral and leaves at the top of the canopy. Conversely, Crawford (2006) did not find differences between treatments at postharvest time during season 2006, but a similar pattern was obtained at veraison and pre harvest.

SPAD measurement has also been found to reflect relative leaf nitrogen status (Porro et al., 2006). However, the amounts of nitrogen analysed in both blades and petioles at flowering were different between treatments but not significant (see Table 4-11, section 4.2.8). Therefore, SPAD values could be related to greater levels of Ca found in blades and petioles collected from shell treatment rather than N amounts, though Porro et al. (2001) only found SPAD values correlated to Ca levels in apple tree.

4.3.2 Canopy temperature:
Canopy temperatures were recorded in the 2006/2007 season and a segment of the leaf and inflorescence development period prior to flowering, from 22nd October to 1st November has been analysed. Data loggers were located in a Stevenson-type screen tied to the first foliage wire, about 100cm above ground level. Figure 4-11 shows temperatures calculated as the difference of Shells minus Control value. The fruit over the shells was in an environment heating up more slowly than in the control in the early morning (up to a -0.5°C difference), but during the day, especially after solar noon, the temperature was 1°C or warmer some times for more than 12 hours, until the after sun
set. The lack of difference in the left of the graph indicates the effect of rainfall, which tends to equalise temperatures (temperature decreased from about 15 to 7°C in this period). A similar effect was described during rain events in the 2004/2005 season (Crawford, 2006).

However, considering the temperature data collected between the 1st November and 31st March (harvest date), there was no statistically significant difference between treatment for mean temperatures (16.7°C versus 16.6°C for Shell and Control, respectively). Likewise over the same period, there were 79 more growing degree days (GDD) in the shell treatment (Table 4-16), being not statistically significant with a mean GDD per day of 8.4 for Shell (sed=0.32) and 7.9 for Control (sed=0.33). In addition, no differences between treatments were found from flowering to harvest (9.0 for Shell, sed=0.35; and 8.57 for Control, sed=0.36), as well as between veraison and harvest date, with the mean GDD per day being 8.1 for Shell (sed=0.65) and 7.7 for Control (sed=0.66).
Table 4-16. Temperature accumulation in the fruited zone, expressed as growing degree days for different periods. No statistical differences were obtained.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>100% Flowering to Harvest</th>
<th>100% Veraison to Harvest</th>
<th>5th leaf unfolded to Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>934</td>
<td>209</td>
<td>1183</td>
</tr>
<tr>
<td>Shell</td>
<td>981</td>
<td>219</td>
<td>1262</td>
</tr>
</tbody>
</table>

¹ 13th December 2006 to 31st March 2007  
² 5th to 31st March 2007  
³ 2nd November 2006 to 31st March 2007

Average hourly temperatures at flowering are presented in the Figure 4-12. It can be seen in that the fruit over the Shells were in a slightly cooler environment at night, after 8 am they were warmer than the fruit in the Control area until after sunset (about 8:30 pm).

![Average hourly canopy temperature at flowering](image)

Figure 4-12. Average hourly canopy temperature at flowering (from 24th November to 14th December 2006). Control treatment (blue line) and the difference of Shells value minus the Control value (red line) are showed with zero being at the right-hand y-axis.

However, the net difference in terms of the average hourly temperature for the whole flowering period was not significant, showing values of 15°C for Shell and 14.6°C for Control, with confidence intervals of 0.38 and 0.34, respectively. Although there were no significant differences in the canopy temperature during the flowering period, the average hourly as well as average daily temperatures followed a different trend during the day time (Figure 4-12) and also during the whole period looking at temperatures each day (Figure 4-13).
In the same way, the average canopy temperatures were very similar during veraison (in February), recording 18.6°C for both shells and control areas (with confidence values of 0.30 and 0.27 respectively). The difference was down as far as -1°C when the temperature was lower in the shell area, due to the temperature rising more slowly in the morning, rather than it being colder and detrimental to grape development (Figure 4-14). In consequence, it could be suggested that shell mulch would not be affecting either mean canopy temperatures at veraison nor growing degree days accumulated from veraison to harvest.
Figure 4-14. Differences of canopy temperatures at Veraison (from 1st to 28th February, 2007).

Figure 4-15. Canopy temperatures at ripening period (average temperature through the day for March).
The average hourly temperatures were also investigated during the ripening period (Figure 4-15). Fruit over the Shells was also in a slightly cooler environment in the morning, but for shorter time than during flowering. Temperatures rose rapidly after 8 am and Shells end up being warmer than the fruit in the Control areas until the after 6:30 pm. Therefore, fruit in the Shells treatment experienced a slightly more continental climate, where extreme temperatures were lower and higher than in the control area. However, the average temperature in March showed no difference between treatments, recording 16.5°C for shell and 16.4°C for control (with sed 0.32 and 0.30 respectively). Canopy temperatures were also measured post harvest from April to the end of July. On average for the whole postharvest period, the fruiting zone temperature tended to be slightly warmer in the Shell treatment but not significant, recording 9.3°C compared to 9.1 °C reached in the control area (with confidence values of 0.15 in both cases). However, the difference was up to -0.5 °C cooler for shell area in some instances, again as a pre-dawn low temperature spike (see Appendix C) also described by Crawford (2006).

Overall, the Shell treatment had no a significant effect on fruiting zone temperatures in the 2006/2007 season, showing similar GDD and average temperatures in both areas over the entire season. However, a different trend was found in 2004/2005 season from 18th November to 30th April 2005 indicating not only 33.5 growing degree days greater in the Shell area over the same period, but also significant differences in average temperature (Crawford, 2006). Clearly, this variation of the data could be related to different weather conditions developed in both seasons, suggesting that probably the 2004/2005 season was cooler than the 2006/2007, which could be confirmed by looking at several differences of temperature from Control, being much lower (up to -2°C) throughout the season (Crawford, 2006). It is possible that the Shells may have a more significant effect in cooler years as opposed to warmer ones.

Under frost conditions (Figure 4-16), canopy temperatures in both treatments react in a similar way. Both areas increased in temperature from around 10am, but the air temperature over the Shell mulch warmed more quickly compared to the Control. A similar tendency was found in the season 2006 (Crawford, 2006). Overall, the use of Shells does not appear to increase the risk of damage by frost.
Figure 4-16. Canopy temperature under frost condition (26-27th June 2007).

Figure 4-17. Canopy temperatures for control compared to the difference between shell and control during the whole season 2006.
In looking at the 2005/2006 season, shells decreased the temperature on average approximately -0.2°C compared to the control over the entire season (October to March), although this was not statistically significant. Shell treatment was cooler for most of the days, even up to 0.6°C cooler in some instances, but slightly warmer at the beginning of the season and also for a period between 13th and 21st December (Figure 4-17). This could be possibly due to warmer season resulting in less impact of shells on influencing temperature. High ambient temperature could mask a treatment difference, as well as the influence of the prevailing winds.

Clearly, the seasonal climate defines environmental characteristics such as average temperatures for the whole period and growing degree days needed for grape maturity, which were not modified by the use of shells. However, fruiting zone temperatures that were slightly higher over the mulch during the day and cooler at night have been seen for all seasons monitored in this trial.

Solar radiation, in conjunction with wind velocity, have been indicated as determinants of fruit temperature, where long wave radiation (UV) was the main source of warming and heat was transferred away by convection (Smart and Sinclair, 1976). Light exposure and berry temperature have been correlated positively during the late afternoon, especially for grapes on the north side of the vine in the southern hemisphere (Lee et al., 2007). Even leaves and fruit exposed to the sun have been 5 to 10 °C higher than those shaded (Kliewer and Lider, 1968). However, despite the fact that air temperature clearly may affect berry temperature, results obtained in the shell trial would indicate that radiation is having the largest impact in influencing the berry environment rather than air temperature around the canopy. Radiation is also affecting temperature, but in a comparatively minor degree. In fact, it has been suggested that due to greater net radiation loss by exposed berries at night, their temperature would be less than shaded berries (Crippen and Morrison, 1986).

On the other hand, sunlight and temperature not only affect photosynthesis and grape growth, but also berry composition and metabolism, influencing total soluble solids, pH, titratable acidity, anthocyanins, phenolics and flavours (Bergqvist et al., 2001; Spayd et al., 2002; Lee et al., 2007). On shell trial, total soluble solids were altered only during the ripening period, being similar for both treatments at the end of the season. Titratable acidity in shells treatment grapes was higher than control, similarly as was reported in a
previous study about the effect of temperature on Merlot (Spayd et al. 2002). This has been attributed to increased malic acid degradation due to higher temperatures of exposed fruit (Lakso and Kliewer, 1978), contrary to what was found in this study: malic acid concentrations were greater in grapes coming from the shells area (see section 4.1).

Given the temperature regimes found in this trial, total anthocyanin concentration did not increase as has been found in previous studies under low night temperatures (Mori et al., 2005). In fact, one to three weeks after the onset of colouring (stage III) is the most sensitive period for anthocyanins accumulation, in conjunction with high levels of abscisic acid and anthocyanin biosynthetic gene activity (Yamane et al., 2006). Temperatures around 35ºC have been reported as reducing total anthocyanin content (Mori et al., 2007), and grapes under warm day (25 ºC) and cool night (15 ºC) conditions developed less colour than those under cool day and night temperatures (both 15 ºC) (Spayd et al., 2002).

4.3.3 Light measurements:
Solar radiation is an important factor influencing growth and ripening of grapes. However, it seems to be that both visible and UV radiation are involved in fruit composition and wine quality (Crawford, 2006). During the 2006/2007 season, reflected radiation was measured in the trial over the flowering period. Sensors for photosynthetically active radiation (PAR), UV-A and UV-B radiation were used to determine the incident and reflected radiation as affected by the treatments. The sky was mainly clear for the time measurements were taken, with only very light high clouds coming in front of the sun for part of the day. Figure 4-18 shows reflected energy in the shell area, with measurements taken at two heights, 25cm from the ground surface and at fruiting wire (FW, 90cm) height. All measurements were taken just off-centre from the row.

Because the sensors' field of vision had a greater amount of vine-row ground covering in it at 25cm height, there was a greater percentage of reflected energy than at FW height. However, the relationship between the treatments was the same, being shells significantly higher in reflected light and UV radiation. In the Control 14% and 8% of incident light was reflected back to the sensor at 25cm and FW height, respectively, while the corresponding numbers in the Shell treatment were 31% and 19%.
Figure 4-18. Percentage reflected energy in the trial compared to bare soil (control) taken on 14th December 2006. Data from two heights are presented: 25 cm from the ground and at the fruiting wire (FW) height. PAR and UV-A radiations were calculated as ratio of reflected over incident energy measured by both sensors. UV-B levels consisted in the average values of two sensors facing up and down, measuring incident and reflected energy.

Height made little difference to the relative increase in reflected light or UV-A, but had a greater effect with UV-B, possibly due to the greater scattering of this shorter-wavelength energy, especially in cloudy days (Mims and Frederick, 1994). Reflected light was approximately two times greater, and UV-A about five times greater in the Shells compared to the Controls. UV-B was similar to UV-A, with three to four and a half times the reflected energy of the Control, depending on the height (Figure 4-19). A similar trend was also found by Crawford (2006).

Measurement of photosynthetically-active radiation (PAR) was also carried out as well as UV radiation at ripening stage in 2006/2007 season. Shell mulch had a positive effect on the amount of reflected UV radiation and PAR into the canopy under sunny conditions (Figure 4-20). In the shell area, the reflection consisted in 64% and 12% of the incident UV-A and UV-B respectively, compared to 2.5% and 4% in control. On the other hand, 54% of PAR was reflected from the Shell mulch, compared to 6% from the control.
Figure 4-19. Relative amount of reflected energy, Shells over Control treatments in December 2006.

Figure 4-20. Percentage of reflectance of the UV and PAR radiation at ripening stage, under sunny conditions. Measurements were taken on 7th March 2007.

The overall pattern of UV radiation was similar in both flowering and ripening stages, being higher in the shell area. However, although UV-B was reflected at the same percentage in both stages, the weaker energy UV-A was reflected 25 times higher in shell area than control, when comparing ripening stage to flowering stage.
The effect of shell mulch in reflecting light was also influenced by the weather. Figure 4-21 shows that under overcast or sunny conditions there was a significant increase of reflected UVA and UVB radiation in the shell treatment, being about 8% in sunny conditions and 12% in overcast conditions.

These results demonstrate that Shell treatment modifies the fruit environment and that the most significant effect may be achieved in cloudy days due to a higher scattering of UV radiation (Pfister et al., 2003; Mims and Frederick, 1994). In fact, the greatest variability in surface UV radiation is due to cloud cover (Lubin et al., 1998).

Sunlight influences berry growth and composition developing a light microclimate into the fruiting zone. However, it is not clear if visible light or UV or both affect fruit composition (Keller and Torres-Martinez, 2004). In fact, incident PAR increased berry temperature on the south side (afternoon exposed) of the canopy by 3-4°C greater than the north side (afternoon shaded), decreasing soluble solids, though TA declined under higher sunlight exposure (Bergqvist et al., 2001). So, PAR radiation could be varying °Brix during ripening period and altering TA and pH.

The amount of light intercepted by vines affects the photosynthetic capacity, water balance and carbon partitioning between vegetative and reproductive growth (Castelan-Estrada et al., 2002). Light environment is evaluated by the total intensity, including the
wavelength band range from PAR (400-700 nm) to UV-B (280-320 nm) (Kataoka et al., 2004). The whole range of wavelengths reflected from shells was considerably higher than control in this trial.

UV radiation influences plant growth, development, morphology and physiology (Keller and Torres-Martinez, 2004) and its effects have been described in both grapes and leaves. The response to UV-B radiation at the plant’s organ and cellular level is mainly an increase in the formation of UV-absorbing compounds to decrease UV-radiation penetration into the tissue (Tevini, 1996), flavonoids being the most effective compound produced in grape leaf and berry tissues (Kolb et al., 2001; 2003).

In grapes, UV radiation can increases anthocyanins (Kataoka et al., 2004), although no effect on anthocyanins and hydroxy-cinnamic acids have also been reported (Keller and Torres-Martinez, 2004). This latter study confirmed that flavonol content notably increases in ripening, post veraison, berries (particularly quercetin-glycoside derivates), but no differences have been observed in proanthocyanidins and catechins (Lafontaine et al., 2005). However, high concentrations of flavonols found in the absence of UV suggest that they also could have a protective function against visible radiation (Keller and Torres-Martinez, 2004). Both treatments in this trial resulted in no difference in the total phenolics concentration measured in grapes and wine, but the amount of individual wine phenolics was altered (see section 4.7). At the same time, total anthocyanins in grapes and wines were similar between treatments in season 2007, though Crawford (2006) found that they accumulated at higher concentration earlier in the season in grapes growing in the shell treatment.

In leaves, UV radiation can also increase leaf carotenoids and flavonoid concentration (Keller and Torres-Martinez, 2004), especially flavonols in leaf epidermis and cuticular wax (Jansen et al, 1998), which are specifically enhanced by UV-B, whereas high visible radiation is associated with the accumulation of hydroxyl-cinnamic acids (Kolb et al., 2001). Similarly, some differences were found in leaf phenolics composition at flowering, although it is still unknown which compounds were varied in this trial (see section 4.7).

Other studies have been carried out previously establishing that UV light has an effect on carotenoid synthesis in developing berries (Steel and Keller, 2000) and main aroma
precursors for norisoprenoid compounds in grapes (Razungles et al., 1993). However, carotenoid concentration is usually higher in shaded than in exposed berries (Bureau et al., 1998) and has been shown to decrease under UV exposure (Schultz, 2000). Analysing aroma compounds through GC methods during grape ripening and final wine would help to clarify this situation.

On the other hand, damage to DNA and alterations in photosynthesis and growth have been cited as negative effects of UV-B radiation (Jansen et al., 1998). UV-B radiation reduced chlorophyll in grapevines (Nunez-Olivera et al., 2006) and leaf senescence occurred faster under UV, decreasing the total chlorophyll in both leaves (as found in this study, see section 4.3.1) and berries, where the damage to the photosynthetic apparatus depended on UV intensity and exposure time (Lafontaine et al., 2005). In a potted Chardonnay study there was an effect of UV on leaf chlorophyll content when measurements were made near to bloom (Keller et al., 2003). Contrary to these studies, SPAD values in this trial were much greater in the shell area; therefore it would seem that the UV-B levels have not been sufficient to alter photosynthesis and other physiological processes in grapevines, or changes in the environment promoted by greater levels of UV-A and PAR were enough to counter UV-B effects.

4.4 Soil environment

4.4.1 Soil temperature
During the 2006/2007 season, soil temperatures were recorded prior to flowering (Figure 4-22). The shells had a large influence on the temperature of the soil at 10cm depth, with the range under shells being about 2°C and that in the Control up to 11 or 12°C on a sunny day. Temperatures fluctuated significantly less in the shell than under the control treatment, which could be influencing positively the shallow root growth.

In fact, Crawford (2006) found a higher percentage of fine roots and lateral branching of roots under the Shell treatment. It is possible that the daytime temperatures were slightly exaggerated in the Control treatment as more of the surface of the soil probes was in sunlight compared to the Shells treatment. However, over this period of 10 days, mean soil temperature in shell area was lower than control at p<0.05, being 15.5°C and 16.6°C, respectively (p=0.016; sed 0.26).
These results would confirm that the use of reflective mulch has a buffering effect on the soil temperature (Mundy and Agnew, 2003; Coventry et al., 2005; Crawford, 2006), though other types of mulch such as straw has also maintained a more constant soil temperature (Van Huyssteen, 1988). Soil temperatures fluctuate less at deeper depths, allowing a continuous root production as occurs in some woody species (Lyr and Hoffman, 1967). Similarly, a buffering effect on soil temperature at shallower depth could suggest a greater root development, though it has been stated that soil moisture retention would have a real effect on root proliferation in the upper soil profile (Smart et al., 2006).

Likewise, shell soil was significantly cooler at 10cm depth in season 2004/2005, considering a longer period of monitoring, from 18<sup>th</sup> November 2004 to 26<sup>th</sup> January 2005. These results are in agreement with other studies which reported soil temperatures lower by 1-3°C (Spratt et al., 2007; Vanden Heuvel et al., 2007) and up to 5°C (Creasy and Nicol, 2003) under a the same reflective groundcover “Extenday”.

It has been reported an effect of warmer root temperatures on greater fruit set in Sultana (Woodham and Alexander, 1966) and with only large changes to soil temperature in Sauvignon blanc (Creasy et al., 2002a), which was not duplicated in this shell trial.
(Figure 4-24). Gladstones (1992) and Kliewer (1975) suggest that soil temperature influences hormonal activities in roots, especially cytokinins. According to Mullins (1967), greater cytokinins content associated at high root temperatures are linked to flower formation and fruitset as well as may play an important role in regulating budbreak and root and shoot growth (Skene and Kerridge, 1967; Zelleke and Kliewer, 1980). So, cooler mean soil temperatures recorded in shell treatment compared to control could be an explanation for poorer fruit set obtained in the shell area during 2006/2007.

Figure 4-23 shows the effect over an average 24 hours between 16th March and 31st March 2007, prior to the harvest (Shells block minus Control). Soil in the shell treatment was slightly warmer than in the control from about mid-night to mid-day and practically the whole night, but for the rest of the day was cooler up to -2.5°C. Similar effects were found during 2005/2006 season (Creasy et al., 2006b), where mussel shells had an insulating effect on the soil, preventing as much solar energy being stored during the day, but also preventing some loss of heat during the night. Any impact from the production point of view is most likely minimal, as these differences are not great enough, nor affecting enough of the root-soil volume of the vines, to change productivity significantly. In other research using plastic and polystyrene mulches, similar trends were found during the day and at nights in November (Creasy et al., 2002b) and early September, but in the Northern hemisphere (Coventry et al., 2005).

![Typical hourly soil temperature at ripening](Figure 4-23. Typical hourly soil temperature during the 16th March 2007 (a day at ripening) at 10 cm depth.)
Similar trends of less variability in soil temperature was seen at ripening stage and even at post harvest time in shell treatment (see Appendix D). However, the average temperatures between treatments were not different at ripening (18.1 for Control and 17.5 for Shell, with p=0.015; sed 0.13; n=3) and post harvest (10.1 for Control and 10.3 for Shell, with p=0.11; sed 0.13; n=3) from 1st April to 26th July. Grapes ripened 10-15 days earlier when soil temperature increased in a mulched soil over the bare control soil (Chkhartishvili and Bekauri, 1979).

In looking at the data separated into two segments of the 2005/2006 season, Figures 4-24 and 4-25 show the difference of Control minus Shells soil temperatures for the periods from 20th September to 31st December 2005 and 1st January to 13th April 2006, respectively. These results confirmed the trends as reported in a previous season (Crawford, 2006), in which the Control soil temperature was consistently warmer than the soil in the Shells treatment through both periods.

![Soil Temperature Differences](image)

Figure 4-24. Differences of soil temperature (Control minus shell) from September to December 2005.
The biggest differences in soil temperatures between treatments occurred in December, and gradually decreased as the season progressed, which was similar to results obtained in another trial using plastic mulches in Canterbury (Creasy et al., 2002a), where the drop in soil temperature at the end of March was associated to heavy rainfall. A similar situation can be seen in Figure 4-25, where soil temperatures consistently fall from 23rd of March 2006, and also in the Appendix D after 1st April 2007. This could be related to clay soil, which may accumulate more water and affect soil temperature and moisture content as it was reported by Tesic et al. (2001), who associated soil texture, rainfall, air temperature and soil depth with vine growth and fruit quality attributes modelling a “site index”.

Soil management techniques should provide the ideal environment for root growth to occur as this is essential for vine growth and productive yield (Richards, 1983). The same author indicates that root growth in grapevines usually takes place when temperatures are above 6°C and has its optimum at 30°C.

Root temperature influences budburst, shoot growth and fruit composition in a controlled environment (Woodham and Alexander, 1966; Kliwer, 1975; Gladstones, 1992). Reflective mulches affect soil temperature as well as light and heat in the canopy (Igounet et al., 1995). However, this research demonstrates that shells reduce soil
temperatures, but the impact of its buffering effect cannot be discounted. Despite several studies reporting about the effect of soil temperature on vine phenology through the use of mulching, it would seem to be that the light reflected from shells is modifying the canopy environment in a greater way. At the same time the slight decrease of temperature found in this trial appears not to be affecting negatively any vine development such as budburst, canopy growth and ripening, with the possible exception of fruit set. Soil temperature is unlikely to have a significant effect on vine performance, given the results obtained by Creasy et al. (2002a). They reported an effect on fruiting, but only with very large changed to soil temperature, which were not duplicated in this trial (Figure 4-22 and 4-24).

**4.4.2 Weed development**

Weed growth was assessed between 16\textsuperscript{th} and 23\textsuperscript{rd} November considering numbers and area (expressed in cm\textsuperscript{2}) covered for each weed type and in both treatments. Overall, the mean total area covered per bay was higher in the control than shell treatment (36,432 cm\textsuperscript{2} and 31,026 cm\textsuperscript{2}, respectively). However, the mean weed number per bay was higher in the shell than control treatment (146 and 114, respectively). It was also recorded that clover coming from the inter-row area encroached more in the control than shells treatment. Thus, weeds were classified according to their growing class in annual, perennial and those that do not fit logically in either category.

<table>
<thead>
<tr>
<th>Weed number</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual</td>
<td>A-P</td>
</tr>
<tr>
<td>Control</td>
<td>511</td>
</tr>
<tr>
<td>Shell</td>
<td>654</td>
</tr>
<tr>
<td>Total</td>
<td>1165</td>
</tr>
</tbody>
</table>

The majority of weeds were classified as annuals in both areas, but the number was higher in the shell treatment (Table 4-17). On the other hand, the total area covered for annual weeds was similar between treatments and perennial weeds in shell treatment covered the double area compared to the control (Table 4-18).

However, when clover was included to analyse the data, it was possible to see that weeds covered a higher area in the control treatment (218,591 cm\textsuperscript{2} versus 186,157 cm\textsuperscript{2} in shell
area). This indicates that shell mulch was effective in keeping out clover from growing area under vines.

Table 4-18. Total area covered for weeds according to their class. Clover is not included.

<table>
<thead>
<tr>
<th>Area covered (cm²)</th>
<th>Annual</th>
<th>A-P</th>
<th>Perennial</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47820</td>
<td>3742</td>
<td>11105</td>
<td>62667</td>
</tr>
<tr>
<td>Shell</td>
<td>48988</td>
<td>1981</td>
<td>20084</td>
<td>71053</td>
</tr>
<tr>
<td>Total</td>
<td>96808</td>
<td>5724</td>
<td>31188</td>
<td>0</td>
</tr>
</tbody>
</table>

Analysing some weeds individually, clover was the more common specie present in both treatments, being higher in the control area. *Malva* sp. (Mallow) and *Anagallis arvensis* (Scarlet pimpernel), both classified as broad leaf annual weeds, were also found more frequently in the control area. On the other hand, *Capsella bursa-pastoris* (Shepherd's purse) and *Veronica persica* (Scrambling speedwell) were recorded in greater numbers in the shell treatment. These results agree with another report where mallow was ranked as the most frequent weed found in vineyards in Nelson and Marlborough, followed by fathen, clovers, redroot and grasses (Dastgheib and Frampton, 2000).

Some weed species were localized only in a particular area (Table 4-19). Of these, Storksbill (*Erodium cicutarium*) and Annual poa (*Poa annua*) had the greater recorded in shell area, whereas Narrow-leaved plantain (*Plantago lanceolata*) and groundsel (*Senecio vulgaris*) were the largest within the control treatment.

Table 4-19. Weeds species localised only in shell or control area according to their growing class.

<table>
<thead>
<tr>
<th>Annual</th>
<th>Perennial</th>
<th>A-P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shell</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild radish</td>
<td>Creeping butter cup</td>
<td>Narrow-leaved plantain</td>
</tr>
<tr>
<td>Storksbill</td>
<td>Indian doab</td>
<td>Onehunga weed</td>
</tr>
<tr>
<td>Annual poa</td>
<td>Turf speedwell</td>
<td></td>
</tr>
<tr>
<td>Clammy goosefoot</td>
<td>Prostrate amaranth</td>
<td></td>
</tr>
<tr>
<td>Bitter cress</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bristle grass</td>
<td>Cat sear</td>
<td></td>
</tr>
<tr>
<td>Scotch thistle</td>
<td>Parsley dropwort</td>
<td></td>
</tr>
<tr>
<td>Bromus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual mouse-ear chickweed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Although there was not weed data collected before the trial was established, these results indicate a shift in weed species between treatments, which could have implications on the management of weeds in the longer term. Different weed control methods such as herbicides, cultivation and mulches have been considered to manage a desired level of weeds (Pool et al., 1990). Herbicide accumulation in the soil, which could damage vine roots, contaminate irrigation dams and leach into the ground water have been reported as possible long-term problems through the use of herbicides (Lennartz et al., 1997). While chemical weed control has showed a trend toward less dependence on residual herbicides such as simazine since 1994 for Nelson area, glyphosate continue being the most common herbicide used in vineyards followed by amitrole, glufosinate ammonium and paraquat/diquat (Dastgheib and Frampton, 2000).

The chemical weed management at Neudorf vineyard consisted in glyphosate applied in the middle of September, and December depending on the season. Buster (glufosinate ammonium) is sprayed in January. Clovers and mallow are tolerant to glyphosate and its repeated use has caused an increase of these weeds on the ground (Dastgheib and Frampton, 2000). In fact, these two plant species were found in greater amounts in the control (bare soil) versus the shell.

A proper rotation of herbicides with different modes of action is a key to avoiding a shift in weed composition or the evolution of resistant weeds. Although no case of resistance has been reported in New Zealand, some species are becoming more tolerant to the most-used herbicides (Dastgheib and Frampton, 2000).

However, appropriate ground management practices are essential to develop a sustainable production system (Pimentel et al., 1992). Non-chemical weed management practices such as cover crops between rows and even under vines (Tesic et al., 2007; Hostetler et al., 2006), winter grazing and cultivation to increase vineyard soil temperature and reduce the risk of frost were described in this study (Dastgheib and Frampton, 2000). The use of mulching also has contributed to the control of weeds in several studies, demonstrating that its use has other advantages at the same time such as increased soil organic matter, increased earthworm activity, and increased water holding
capacity. Periodic replenishment of the shells may help to keep weed populations down, though the effect of this practice has not been tested.

4.5 Berry ripening

4.5.1 Veraison monitoring:

Veraison was defined as the process of colour change for the colour change progression results. The progression of veraison was assessed between 19th February and 7th March during the 2006/2007 season using the visual scoring system (see Table 3-1, section 3.4.3.4). Change of colour was monitored and grapes in the shell area were slightly advanced compared to grapes in the control, especially from 26th February, in which the values were statistically significant (Figure 4-26). Thus, veraison was completed three days earlier in the shell area.

![Progression of colour at Veraison](image)

Figure 4-26. Progression of colour change at veraison in 2007. Bars indicate standard errors of treatment means (n=90, Control; n=89, Shell).

Early veraison can mean an advancement of the ripening, which could be seen in the data collected during berry sampling, where shell clusters were slightly higher in °Brix compared to those from the control area in the middle of the sampling period (see Figure 4-29; Crawford, 2006). Veraison date usually varies from year to year (Coombe, 1992). By using reflective mulch, veraison stage was advanced (in colour and sugar level) in bagged as well as non-bagged clusters, suggesting that the advancing of maturity did not
occur only through light incidence on the clusters (Coventry et al., 2005). Other, related factors are discussed in section 4.5.2.

4.5.2 Berry colour variability at veraison

Colour variability in the bunch was assessed on 21st February 2007 (at 90% of veraison completed), visually classifying individual berries on clusters according to four different degrees of colour: green, pink, red and blue. The colouring stage indicates the degree of progression through veraison.

Figure 4-27 shows the proportion of different berry colours based on the total number of berries in a cluster. The majority of berries were classified under the blue colour in both treatments, and the proportions of red, pink (those which were just showing colour) and green berries were also not statistically different between shell and control. These results are in agreement with the values scored in both areas on 21st February when veraison was visually ranked and no differences between treatments had been observed. However, score values obtained later were statistically different in the colour progression between 27th February and 2nd March (Figure 4-26). These results could indicate that shell mulch may contribute to a decrease in the variability of colour within the clusters at veraison and that this stage could complete sooner than Control. In fact, a slight decrease in berry variability within the shell clusters at harvest 2006 was found, where the majority of berries contained similar °Brix level (see section 4.1.2).

Figure 4-27. Berry variability according to the colour at 90% of veraison completed (21st February). Bars indicate standard errors of treatment's proportion means (n=3016).
In a cool climate, a later veraison can affect fruit ripening due to the potential for unfavourable weather conditions nearer to winter (Smart et al., 1988). Weather conditions were favourable for the veraison stage in 2006/2007 season. February was a sunny month with 249 hours of bright sunshine and drier with just 7.5mm on four days compared to the usual rainfall of 61.5mm. The mean temperature was 18.6°C with the warmest day being 27.1°C and the coolest night 10.9°C.

4.5.3 Crop estimation and berry size distribution

Predicted yield was assessed at 90% of veraison completed (21st and 22nd February). Several variables from the 12 bunches per treatment were recorded such as cluster weight, wing weight, rachis weight and total berry number. All yield components were found to be statistically different between treatments, except rachis weight (Table 4-20). Values of cluster weight, wing weight and cluster weight with no wing were higher in bunches from the Control area compared to those coming from Shell, whereas berry number was greater in Shell treatment than Control.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cnt Average</th>
<th>Cnt St Dev</th>
<th>Shell Average</th>
<th>Shell St Dev</th>
<th>Confidence Cnt</th>
<th>Confidence Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster weight</td>
<td>102.9</td>
<td>18.4</td>
<td>66.2</td>
<td>12.4</td>
<td>10.4</td>
<td>7.0</td>
</tr>
<tr>
<td>Wing weight</td>
<td>38.3</td>
<td>13.8</td>
<td>17.1</td>
<td>10.4</td>
<td>7.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Cluster weight no wing</td>
<td>64.6</td>
<td>10.5</td>
<td>49.0</td>
<td>9.2</td>
<td>5.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Berry number</td>
<td>91.9</td>
<td>27.9</td>
<td>159.4</td>
<td>24.6</td>
<td>15.8</td>
<td>13.9</td>
</tr>
<tr>
<td>Rachis weight</td>
<td>4.3</td>
<td>1.1</td>
<td>5.4</td>
<td>0.8</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

It has been proposed that a factor of 1.3 applied to veraison cluster weights in New Zealand gives a reasonable estimation for the harvest bunch weight, thus predicting yield (Crawford, 2006). On the basis of flower cluster number at post shoot thinning (see section 4.2), a higher yield in the control area was predicted, but cluster weight was higher in the control treatment as well, leading to an estimate of substantially more crop per vine in the control area (Table 4-21).

<table>
<thead>
<tr>
<th></th>
<th>Est. mean clusters/vine</th>
<th>Sample mean cluster wt (g)</th>
<th>Est. mean wt harvest cluster</th>
<th>Est. Kg/vine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.2</td>
<td>102.9</td>
<td>133.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Shell</td>
<td>26.6</td>
<td>66.2</td>
<td>86.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 4-20. Yield components taken at 90% veraison completed (22nd February 2007) from 12 bunches in each treatment. Confidence values at 95%.

Table 4-21. Yield estimation from trial site, February 2007.
To find out more about variability within the clusters at this stage, berries also were grouped according to three sizes and then weighed by group, getting three mean weight classes per berry: <0.4 (small), 0.5 to 0.7 (medium) and >0.8g (big). Berries in wings were not considered for this study. The distribution of berries in these weight classes is presented in Figure 4-28.

![Berry distribution according to size](image)

Figure 4-28. Berry distribution according to the size defined by berry weights at 90% of veraison completed (21st February). Error bars are standard errors of treatments proportion means (n=3016)

As it can be seen in Table 4-20, there were a greater total number of berries in clusters coming from shell treatment in comparison with the control (159 and 92, respectively). However, despite this difference, a greater proportion of berries in shell clusters were classified as the smallest weight class (seedless berries), representing 88% of the total berries in the cluster. In contrast, similar percentages of berries almost representing 1/3 were classified in each weight class in control clusters. This is a significant change in berry size distribution as result of the shells treatment, and has implications not only for yield, but also wine characteristics.

Similar to the 2004/2005 season, a reduction of cropping level over the shell area was seen in 2006/2007 season (see section 4.6). A poorer seeded fruit set in the shell treatment area caused a higher number of smaller berries on clusters (seedless berries) with the added effect that some of them shrivelled during ripening period. This could result in a decrease of berry weight and consequently lower yield, as was seen by Crawford (2006). Although lower yield in the shell area had been predicted at veraison in 2006/2007 season, the values obtained at harvest were much lower than had been
expected (see section 4.6). However, a higher crop load had been estimated for 2004/2005 season in shell area which was completely opposite at harvest that season (Crawford, 2006). In addition, it seems to be that the multiplication factor of 1.3 was not correct in this location and season for an accurate prediction of yield.

While total flower number is the most critical variable contributing to yield, berries per bunch is the second most important factor after bunches per vine (Martin et al., 2000) and can be responsible for 30% of seasonal variation in yield. Petrie and Clingeleffer (2005) suggested that the variation in flower number would vary berry number, and variation in flower size, would translate into final berry size. However, Friend (2005) demonstrated that flower size does not determine berry type, although there could exist some effect of environmental conditions on influencing the success of fertilisation.

Several studies have shown that increases of temperature in different stages over the season may result in greater berry number and berry weight. For example, it has been cited that increasing bud temperature during budbreak promotes a greater berry number and berry weight (Keller et al., 2005). At the same time, productivity was enhanced due to an increase of the average berry weight and berry number in a cluster under reflective mulch established at bloom (Robin et al., 1997). However, berry weight was not affected by temperature at harvest according to Yamane et al. (2006). In addition, a higher mean daily temperature from between 15.5 and 17.5°C can result in an increase in average bunch weight of up to 60% (McGregor, 2000). It seems that increases in temperature in the shell treatment may be influencing positively berry number and negatively berry weight, contrary to the negative correlation between temperature and inflorescence flower numbers at budbreak, reported by Dunn and Martin (2000).

Sufficient light is also required for normal berry and cluster development during the season. Exposed and shaded clusters were smaller than those from moderate exposure, because shaded clusters had fewer berries than more exposed clusters, and berry weight decreased as cluster exposure increased (Price et al., 1995). This could explain the lesser quantity, but heavier berries found in the control area. However, a more efficient use of incident light in shell canopies could be increasing berry number per bunch due to higher photosynthesis in the season past, arising from greater photosynthetic photon flux
densities, as suggested by Morgan et al. (1985). But Shell berries were smaller due to a lack of seeds or very small seeds.

Berry growth may be reduced by increased exposure to direct light, particularly if fruit temperatures are elevated further than the optimum for development (Hale and Buttrose, 1974; Bergqvist, 2001). However, similar average berry weights at veraison as well as at harvest were reported from shaded and exposed treatments (Cortell and Kennedy, 2006). High berry temperatures can affect berry cell division or elongation as well as increase fruit transpiration rates and subsequent berry dehydration (Crippen and Morrison, 1986b), which can cause shrivelling in berries. However, over-exposure of grape clusters to both excess solar radiation and temperatures after leaf removal (described by Bondada and Keller 2007) could be the best explanation for shrivelled berries found in the shell treatment, which resulted in lower weights affecting yield. The loss of weight not only is associated with berry shrivel during ripening, but also is related to poor fruit set, which decreased berry weight cluster weight as assessed at veraison. Although Coombe et al. (1987) suggested that berry weight may influence wine phenolic concentration, it has been demonstrated that phenolic concentration in wine is not necessarily related to berry size (Roby et al., 2004). This is in agreement with the results obtained during 2005/2006 season, in which shell clusters showed bigger berries than control but similar phenolics in grapes (see section 4.1), and resulting wines were also similar in total pigments and total phenolics (see Table 4-26, section 4.7). However, although there were mainly smaller berries in shell clusters during 2006/2007 season, the resulting wines were different in individual phenolic compounds (see section 4.7.2.2).

4.5.4 Progression of ripening:

Ripening was monitored from 5th March weekly to harvest date. Figure 4-29 shows the trend of °Brix, TA and pH analyses carried out during ripening period. While control samples increased pH and decreased TA consistently during the four weeks, shell samples shown a slight increase of pH up to 3.09 and a stable TA around 8.7 g/L, being higher than control near to harvest. There were significant differences between treatments when samples were taken in the middle of the period (12th and 18th March). While °Brix was significantly higher in shells at both these dates, pH was greater on 12th March, but lower than control on 18th March.
On the other hand, measurements at the beginning of ripening and one week before harvest had similar trends in both treatments for all of the variables measured in each time, except the higher TA found in shell treatment at the end of the sampling period. However, although the advancement of ripening seen in the shell area was reduced at the end of season 2004/2005, shell had a higher TA compared to control at the beginning of that ripening period (Crawford, 2006).

Sunlight is a factor that influences berry ripening processes and determines the amount of photosynthesis that occurs, due to that carbohydrates accumulated by berries are related to the photosynthetic capacity (Petrie et al., 2000). In addition, limiting sunlight during veraison may delay grape ripening (Keller et al., 1998). While some studies have seen that percent soluble solids are not affected by exposing or shading clusters from sunlight or artificial alteration of temperature (Spayd et al., 2002; Downey et al., 2004; Cortell and Kennedy, 2006), other researchers confirmed that this parameter is higher in exposed clusters (Smart and Sinclair, 1976; Reynolds et al., 1986; Pereira et al., 2006) or lower under fruit shading (Gao and Cahoon, 1994; Reynolds et al., 1996; Price et al., 1995).
Clearly, a variation in sugar levels was expected in this trial due to increases of light and UV radiation promoted by shell mulch.

However, despite the fact that there was a significant change in the canopy environment, °Brix was not different at harvest. This is in agreement with other studies which showed no influence of UV (Keller and Torrez-Martinez, 2004) or changes on reflected light (by using crushed quahog shells and Extenday® mulch) (Vanden Heuvel et al., 2007; Spratt et al., 2007) on sugar content. But, few differences in ripening time and fruit composition at harvest were found by using a white reflective geotextile (Hostetler et al., 2006) or greater levels under improved light exposure by using solarisation (Robin et al., 1997).

Sugar accumulation was only greater in the shell area during the ripening period, contrary to other findings where higher values were measured post veraison by using white coloured foil mulching and at harvest through all coloured (red, silver and white) foils (Todic et al., 2007). Similar results were reported by Coventry et al. (2005) using aluminised polyethylene sheeting.

Although parameters such as pH and TA were unaffected by using different types of reflective mulches (Vanden Heuvel et al., 2007; Spratt et al., 2007), shell mulch had a positive effect on TA at harvest as it was also reported in exposed grapes by Cortell and Kennedy (2006), which could be related to greater malic acid content found in grapes at harvest during 2005/2006 season (discussed in section 4.1.2). Other studies have confirmed that TA declines with increasing sunlight exposure (Bergqvist et al., 2001; Reynolds et al., 1986), which has been attributed to increases of malic acid degradation due to higher temperatures (Kliewer, 1971).

TA and pH may be greater in fruit from high vigour-zones (Jackson and Lombard, 1993), though there also may be a reduction in sugar accumulation (Cortell et al., 2007). Since canopy assessments as well as several measurements related to vine vigour were similar between shell and control treatments, it is unlikely that these factors are affecting this trial.
The effect of light has been also associated to temperature. Berry temperatures typically follow a diurnal pattern than mirrors solar radiation (Spayd et al., 2002) and where temperatures are increased well above ambient (Smart and Sinclair, 1976). So, it could be assumed that increasing sunlight into the canopy would cause rising berry temperatures. Fruit composition was found to be dependent on berry temperature, increasing under sunlight exposure (Bergqvist et al., 2001), though temperatures higher than 37°C measured during ripening period inhibited sugar accumulation (Kliwer, 1977; Haselgrove et al., 2000; Bergqvist et al., 2001). However, no effect of temperature on total soluble solids at harvest has also been reported (Yamane et al., 2006).

°Brix has been reported to be greater under high night temperature conditions at veraison, though similar after veraison when comparing warm and cool nights (Mori et al., 2005). This suggests that the temperature at veraison is critical for sugar accumulation. However, warmer days and cooler nights was the trend in canopy temperatures throughout the whole season in the shell area. Because of this, the variation of different parameters during ripening may be related more to light intensities rather than temperatures in the shell trial.

4.5.5 Peduncle lignification progression

Lignification of the peduncle was assessed at veraison and harvest stage using a scale between 1 and 5 (from green to full dark), rating the advance of the colour from the shoot to the first branch of the cluster. Figure 4-30 shows that at both stages monitored there were significant differences between treatments, comparing the grand mean (3.42) with means obtained from each row. Peduncles observed in the control area were coloured brown in advance compared to those in the shell treatment at veraison as well as at harvest. In addition, peduncles from both treatments increased their lignification at the same rate between veraison and harvest.

°Brix, pH and titratable acidity are valuable tools for assessing ripeness during the season. However, visual indicators such as rachis and pedicel lignification, berry coloration and seed colour allow also evaluating fruit maturity. Particularly, peduncle lignification has been used as a visual parameter to characterize grapevine varieties and Vitis species under the code OIV 207 (OIV, 1983; Masi et al., 2001).
Interestingly, contrary to the wine industry impression who has assumed better tannin maturity in berries with higher peduncle browning, wines made with grapes from browner peduncle into the bunches resulted to be scored lower in phenolic ripeness during the sensory evaluation (see session 4.7.3). However, further research analysing phenolics content in stems related to those in berries during ripening could clarify this idea.

4.6 Harvest and winemaking

4.6.1 Yield

Grapes for microvinification were collected on 27\textsuperscript{th} March 2007 from 8 control bays and 14 shell bays, considering 5 vines per bay. While forty control vines yielded 59.1kg, sixty nine Shell vines recorded 65.6kg. Average yield per bay was higher in the control area compared to shell (Table 4-22). However, the table also shows that the number of bunches recorded after shoot thinning was not different between treatments in these specific bays. This suggests that the difference in yield could be due to a decrease in cluster weight in the shells area during the ripening period.
Table 4-22. Number of bunches (post shoot thinning) and yield gathered from the same bays, which were harvested for microvinifications. Confidence intervals are at 95% and n=90.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg/bay cluster/bay</td>
<td>kg/bay cluster/bay</td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>7.4</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>St Dev</strong></td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Conf. + -</strong></td>
<td>1.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Although yield components such as bunches per vine and cluster weight were not taken directly from each plant, they may be calculated from the data obtained previously (Table 4-23).

Table 4-23. Yield components gathered from the fruit used for 2007 microvinifications.

<table>
<thead>
<tr>
<th></th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Wt/vine (kg)</td>
<td>1.48</td>
</tr>
<tr>
<td>Cluster weight (g)</td>
<td>53</td>
</tr>
<tr>
<td>Cluster # / vine</td>
<td>27.8</td>
</tr>
</tbody>
</table>

1 Values from average kg/bay divided by 5 vines
2 Values from average kg/vine divided by average cluster #/vine
3 Values from average cluster #/bay divided by 5 vines (after shoot thinning)

Average cluster number per vine was similar between treatments. This means that the poorer yield per vine found in shell area could be due to lower average cluster weight, contrary to what was reported in 2004/2005 season, where bunches per vine were lower in the control area at harvest (Crawford, 2006). However, although the average bunch weight was also lower in the shell area when analysed on 26th March (one day before harvest) (Table 4-24), the values were higher in both treatments if they are compared to those obtained from the average yield/vine over average cluster number/vine (Table 4-23). This would indicate that possibly there was a tendency to collect larger bunches during pre-harvest sampling from whole rows.

Table 4-24. Bunch weights of 10 clusters collected at harvest and post veraison from six whole rows. Confidence intervals are at 95%.

<table>
<thead>
<tr>
<th></th>
<th>6/03/2007</th>
<th>26/03/2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell</td>
<td>72.0</td>
<td>61.3</td>
</tr>
<tr>
<td>Control</td>
<td>70.4</td>
<td>93.7</td>
</tr>
<tr>
<td>Mean (gr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St Dev</td>
<td>21.0</td>
<td>23.5</td>
</tr>
<tr>
<td>Conf</td>
<td>13.0</td>
<td>14.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Shell</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (gr)</td>
<td>61.3</td>
<td>93.7</td>
</tr>
<tr>
<td>St Dev</td>
<td>23.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Conf</td>
<td>14.6</td>
<td>16.4</td>
</tr>
</tbody>
</table>

Interestingly, mean bunch weight values found on 6th March did not agree with those seen at veraison, when crop estimation was assessed from just the first 3 bays of the same
rows (22nd February). Higher average cluster weights had been measured in control treatment compared to shell (see section 4.5.3). It seems that there was a high variability in crop loading in the whole block compared to the area designed for this trial. In addition, there was a sampling error involved, collecting larger clusters instead taking a truly random sample from the area, which could explain the variability in the results obtained. However, the decrease of cluster weight between veraison and some days before harvest, according to the data shown in Table 4-24, could suggest berry shrivelling seen in shell clusters at the end of the ripening period.

On the other hand, grapes for commercial wine were harvested on 27th March from the shell and 31st March from the control area, from both the upper and lower areas. Overall, values gathered for yield were similar between shell and control areas (1.24 and 1.29kg/vine, respectively), which is contrary to the reduction of yield seen in the sampling trial. A high variability characterised in the block by Crawford (2006), being upper and lower slope quite different, could explain a balanced yield seen during 2006/2007 season.

Overall, further explanation is needed to confirm if the decrease in yield is associated to a shell effect rather than seasonal influence. While yield in the shell area was greater for microvinifications as well as commercial grapes in 2003/2004 season, shell grapes yielded lower than control for the microvinifications and no differences were found for commercial harvest in 2004/2005 season (Crawford, 2006). However, yield components were not different between treatments for microvinifications harvest in 2005/2006 season, contrary to the 2006/2007 season, where shell grapes yielded less compared to control for microvinifications but unclear for commercial harvest. Clearly, sampling errors involved in predicting yield were made during this season (and possibly in prior seasons, too) and differences in vine growth and production exist considering the block as a whole and the experimental area established for this season.

Increasing reflected light into the canopy could mean greater yield. Vines mulched with white geotextile which increased by 50% light in the cluster zone, had greater yields (Hostetler et al., 2006). However, fruit yield was unaffected by using a synthetic reflective mulch, crushed quahog shells or Extenday® mulch (Vanden Heuvel et al., 2007). It is likely that some particular radiation is influencing cropping level, though UV
has failed to influence yield (Keller and Torrez-Martinez, 2004) and any radiation was measured by Vanden Heuvel et al. (2007). Reflective mulches induce changes in field microclimate (see section 4.3), affecting not only light but also slightly temperature. Early season temperature differences (at budbreak time) could affect reproductive growth, influencing also yield components (Keller et al., 2005). However, it seems that reflected light consisting in PAR and UV radiation is influencing yield in the shell trial rather than temperature slightly modified by same radiations as well.

4.6.2 Must composition

Figure 4-31 shows basic analyses of must post-crushing in both 2005/2006 and 2006/2007 seasons (harvest composition data). By looking at last season, °Brix values were different between treatments, with Shell values being considerably higher than control (approximately 1.5°Brix).

Although there was no difference in pH (around 3.23), TA values were slightly higher in the Shells treatment compared to the Control, but not statistically significantly. However, there is no correlation when these data are compared with 2005/2006 season, where °Brix and TA values were similar between treatments, though pH was statistically greater in shell must compared to the control. On the other hand, Crawford (2006) concluded that
harvest analyses revealed no differences in composition between treatments in 2004/2005 season. These results indicate that there is likely an effect of season conditions on fruit composition, which could result in advanced sugar ripeness in the shell area, with pH and TA values following different trend. The greater °Brix levels in shell musts could be also associated with higher quantities of shrivelled berries and smaller berries found in the shell area, which usually increase concentration of soluble solids, similar to that reported by Keller et al. (1998).

Must for microvinification and commercial wines were tested before fermentation for other parameters (Table 4-25). Despite the fact that single samples were sent to the laboratory (based on one sample), these results are similar to the values found during maturity monitoring and at harvest, where °Brix was higher in shell treatment. Although pH was very similar between treatments in the microvinifications, values were slightly higher (0.25 units) in the shell treatment for commercial wine. It is likely that this was due to the extra time lapsed between sampling and processing.

Table 4-25. Must analyses after 24 hours soaking according to Pacific Rim Oenology Services LTD in 2006/2007 season. No statistics are available. Shell and control grapes for microvinification harvested the 27th March; Shell commercial harvested the 27th March and control commercial the 31st March.

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>Microvin</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brix, refractometer</td>
<td>°</td>
<td>Shell 25.4</td>
<td>Shell 25.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 24.2</td>
<td>Control 23.2</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>Shell 3.35</td>
<td>Shell 3.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 3.37</td>
<td>Control 3.05</td>
</tr>
<tr>
<td>Malic acid, enzymatic</td>
<td>g/L</td>
<td>Shell 2.43</td>
<td>Shell 2.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 2.40</td>
<td>Control 2.95</td>
</tr>
<tr>
<td>Nitrogen, alpha amino</td>
<td>ppm</td>
<td>Shell 222</td>
<td>Shell 214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 190</td>
<td>Control 214</td>
</tr>
<tr>
<td>Nitrogen, ammonia</td>
<td>ppm</td>
<td>Shell 96</td>
<td>Shell 101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 91</td>
<td>Control 78</td>
</tr>
<tr>
<td>Yeast Available Nitrogen</td>
<td>ppm</td>
<td>Shell 318</td>
<td>Shell 315</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control 281</td>
<td>Control 292</td>
</tr>
</tbody>
</table>

Malic acid tested by enzymatic method was slightly higher in the shell musts used for microvinification and slightly lower in the commercial wine musts (by approximately 0.09 g/L). Nitrogen in both ammonia and YAN forms were higher in the shell treatments for microvin and commercial wines in 2006/2007, similar to what was found in the 2004/2005 season (Crawford, 2006) from the same area of the block, though ammonia was only marginally greater in shell microvin. Vine water availability has an important effect on yeast assimilable N in grape juice (Keller and Torrez-Martinez, 2004). So, it appeared that shell is creating a soil environment with greater moisture during the season as it was reported by Crawford (2006) and at the same time it is influencing YAN. This matches results found in petiole and leaf blade analyses, where the percentage of nitrogen found in shell treatment was also higher (see section 4.2).
While alpha amino nitrogen was found to be slightly higher in shell microvin must compared to control, a similar level was reported between treatments in the commercial wine must analysis. This was in agreement with another study where UV radiation did not influence juice amino-N (Keller and Torrez-Martinez, 2004), though contrary to what was reported by Schultz (2000) who suggested that UV inhibits the incorporation of nitrogen into amino acids.

Harvest date constitutes another important point related to winemaking, especially in cool climate regions. While both commercial and microvin grapes were harvested on the 5th April from shell and control area in 2004/2005 season (Crawford, 2006), harvest took place on 16th March in 2005/2006 season. However, microvin grapes from both treatments and commercial shell were picked on 27th March 2007, whereas commercial control was harvested on 31st March in 2006/2007 season. Clearly, grapes in both areas achieved satisfactory ripeness on different dates depending on the season.

### 4.6.3 Wine composition from Microvin

Wine analyses such as alcohols, pH and TA values were taken from each replicate before bottling in both 2005/2006 and 2006/2007 seasons (Figure 4-32).

![Wine Analyses pre-bottling 2007 and 2006](image)

Figure 4-32. Wine measurements taken from microvinifications 2007 and 2006. TA is expressed in g/L and alcohol as % by volume. Bars indicate standard errors of treatment means (n=3).
The results for the 2006/2007 wines were very different when compared to the previous season. Shell replicates had higher alcohol than control wines, which was correlated to °Brix measurements taken earlier in the season (see section 4.5). However, TA and pH in shell wines were not statistically different compared to control, though TA was slightly higher than control. These results agree with solarisation experiments by Robin et al. (1997), who found the reflective treatment resulted in wines with higher alcoholic concentration, though also greater in total acidity.

However, in looking at the wines made in 2005/2006 season, slight differences were found for all parameters, but none were statistically significant. Similar results were reported in pre-bottling analyses assessed in 2004/2005 season (Crawford, 2006). Interestingly, °Brix and TA values measured in 2006/2007 season were considerably higher than those obtained in 2005/2006 for shell and control wines. Overall, percentage alcohol by volume in 2006/2007 season was in the 13.8% to 14.6% range, about 0.8% higher than 2005/2006 season. TA values were in a range between 6.63 and 6.29 for shell and control respectively, being around 0.8 mg/L higher compared to 2005/2006 season. These results could be explained by weather conditions and different harvest dates developed in both seasons, being about 10 days later in the last season.

4.7 Phenolic and aroma composition

4.7.1 Wine Spectrophotometric Analysis

Wine replicates for microvinification of 2006 vintage were tested spectrophotometrically by the method of Iland et al. (2000). Wine pH values had somewhat equalised with three months in the bottle (Table 4-26), and there were no significant differences between treatment wines in the other parameters measured. However, there were much higher than previous vintages values for Total Pigments and Total Phenolics (in the range of 10 to 25 for the 2004, and 5 to 27 for the 2005 microvinifications respectively, data not shown), probably due to a reflection of the vintage and winemaking techniques. Four cap punch downs per day were made during harvest 2006 compared to twice daily (morning and evening) during seasons 2005 and 2007.
Table 4-26. Wine spectrophotometric measurements of the 2006 microvinification wines. Confidence intervals at 95% from 3 replicates.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Shell</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>St Dev</td>
<td>Conf</td>
<td>Average</td>
</tr>
<tr>
<td>pH</td>
<td>3.5</td>
<td>0.038</td>
<td>0.04</td>
<td>3.6</td>
</tr>
<tr>
<td>Wine Colour Density</td>
<td>8.4</td>
<td>0.327</td>
<td>0.37</td>
<td>8.2</td>
</tr>
<tr>
<td>Wine Colour Hue</td>
<td>0.6</td>
<td>0.070</td>
<td>0.08</td>
<td>0.6</td>
</tr>
<tr>
<td>Degree Pigment colouration (%)</td>
<td>0.1</td>
<td>0.009</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>SO2 Resistant Pigments</td>
<td>1.4</td>
<td>0.076</td>
<td>0.09</td>
<td>1.4</td>
</tr>
<tr>
<td>Total Pigments</td>
<td>45.8</td>
<td>2.54</td>
<td>2.87</td>
<td>46.8</td>
</tr>
<tr>
<td>Total Phenolics</td>
<td>126</td>
<td>9.5</td>
<td>10.8</td>
<td>126</td>
</tr>
</tbody>
</table>

Higher values of colour were expected. However, these results confirmed the others found in earlier vintages, where the analytical differences between the wines were minimal (Crawford, 2006). Robin et al. (1997) obtained greater polyphenol content and coloration in wines made with grapes under the effect of aluminised film from the bloom state. Another study reported an increase in UV induced formation of red and brown pigments without affecting sugar under field conditions (Lafontaine et al., 2005), with UV-B radiation responsible for the increased phenolics. The results found for total pigments and phenolics in the trial wines could also be correlated to the lack of differences in whole clusters analysed at harvest during 2006 (see section 4.1).

4.7.2 HPLC analysis

4.7.2.1 Leaf samples

Leaf blade samples at flowering (7th December 2006) and post-veraison (6th March 2007) were analyzed by HPLC-DAD. Similar concentrations of the flavonol rutin were found in shell and control treatments at flowering, with values of 160.7 and 169.7 mg/L respectively (p=0.41; sed= 9.98), with no differences found by comparing peak areas of other phenolics contained in leaves from both areas at flowering and post-veraison. However, areas obtained from leaves collected at flowering were then log transformed (base 10) prior to statistical analysis, as there appeared to be a linear relationship between treatment means and standard deviations from the mean.
As can be seen in Figure 4-33, several unknown phenolic compounds defined by their retention times varied slightly in comparing both treatments. However, the compounds with retention times of 57.6 and 59.3 minutes were the most different between treatments, both being greater in shell wines when they are compared through the overall standard errors (sed=0.13).

These results would indicate that increases of UV radiation and light early in the season could modify phenolic content in leaves, though it was not possible in this study to identify the single compounds. Ambient with high UV levels increased the concentration of flavonols (quercetin-glycoside derivates) in leaves collected at mid-season, but had no effect on hydroxy-cinnamic acids (caffeyl and p-coumaryl tartaric acids) (Keller and Torrez-Martinez, 2004), using the same analysis method described by Keller et al. (2000). In addition, higher UV levels and low nitrogen stimulated accumulation of leaf flavonols, which are located mainly in the epidermis and cuticular wax, acting as a sunscreen for plant tissues (Jansen et al., 1998). Despite the fact that another study (Kolb et al., 2001) used different light regimes provided by foils exhibiting different UV transmission inside the greenhouse, biosynthesis of flavonols in grapevine leaves was
enhanced by UV-B radiation, whereas high visible radiation stimulated accumulation of hydroxy-cinnamic acids. However, UV-A did not affect leaf phenolics (Kolb et al., 2001).

Interestingly, there was no effect of UV and light on phenolic contents when leaves were sampled after veraison. Under field conditions, compounds such as quercetin, isorhamnetin and kaempferol derivatives were detected in leaves of *Vitis labruscana* collected at ripening (Park and Cha, 2003). In addition, leaf proanthocyanidins such as epicatechin and catechin were similar at all stages of leaf development (Bogs et al., 2005). According to these results it could be assumed that UV and light had no effect on these compounds. Further research by using HPLC-MS could clarify these differences.

### 4.7.2.2 Wine samples

HPLC-DAD analysis was applied to microvin and commercial wines from season 2006/2007. The HPLC chromatograms recorded at 280nm showed differences in several peak areas between shell and control microvin wines (see Appendix E and F). These peaks were identified as hydroxycinnamic acids, flavan-3-ols, benzoic acids, flavonols and stilbenes.

![Phenolics and stilbenes in Microvin wines 2007](image)

Figure 4-34. Phenolics and stilbenes in microvin wines 2007. Mean Logs 10 of the concentration are statistically significant at p<0.001 between treatments for all of compounds. Bars indicate standard errors of treatment means (n=3).
However, there were no statistically significant differences between treatments for these compounds (data not shown). Therefore, concentrations were log transformed (base 10) prior to statistical analysis as it was done previously with leaf data. A summary of the resulting analysis is presented in Figure 4-34.

Phenolic compound peak areas identified in microvins according to the standards used were different at p-value <0.001 when they were analyzed by ANOVA. However, only quercetin and resveratrol were significantly higher in shell microvin wines in comparing means (sed=0.08). Flavan-3-ol concentrations such as epicatechin and catechin were not statistically different between treatments, though the values were slightly greater in control wines. At the same time, compounds such as caffeic acid, gallic acid, p-coumaric acid and rutin were also similar between treatments, and only slightly higher in shell wines.

![Flavan-3-ols in Commercial wines 2007](image)

*Figure 4-35. Flavan-3-ols in commercial wines 2007. Mean concentrations expressed in mg/L are statistically significant at p<0.001 for both compounds. Bars indicate standard errors of treatment means (n=3). The concentrations (mg/L) are in catechin equivalents.*

In the commercially vinified wines, flavan-3-ols showed a dissimilar trend, being statistically greater in the control wine (Figure 4-35). However, benzoic acids and stilbenes were also higher in control compared to shell, contrary to that found in microvin
wines (Figure 4-36). Other compounds identified previously in microvin wines such as hydroxycinnamic acids and flavonols were not different between treatments in commercial wines.

![Benzoic acids and stilbenes in Commercial wines 2007](image)

Figure 4-36. Gallic acid (benzoic acid) and resveratrol (stilbene) concentrations in commercial wines 2007. Mean concentrations expressed in mg/L are statistically significant at \( p<0.001 \) for both compounds. Bars indicate standard errors of treatment means \((n=3)\).

Additionally, several other peaks areas of unidentified compounds were statistically different between shell and control microvin wines for 2007 at \( p<0.001 \). By analysing the retention times and peak spectra of these unknown compounds, some of these phenolics were tentatively identified (Table 4-27).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Ret time (min)</th>
<th>Area (uV*sec)</th>
<th>Possible compound</th>
<th>Absorbance peaks in spectrum (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.712</td>
<td>228262</td>
<td>266095</td>
<td>caftaric acid-ester</td>
</tr>
<tr>
<td>2</td>
<td>15.962</td>
<td>204312</td>
<td>247757</td>
<td>unknown 1</td>
</tr>
<tr>
<td>3</td>
<td>27.728</td>
<td>311391</td>
<td>394306</td>
<td>unknown 1</td>
</tr>
<tr>
<td>4</td>
<td>32.362</td>
<td>1191320</td>
<td>1681775</td>
<td>unknown 2</td>
</tr>
<tr>
<td>5</td>
<td>36.128</td>
<td>249696</td>
<td>357230</td>
<td>unknown 1</td>
</tr>
<tr>
<td>6</td>
<td>38.212</td>
<td>82188</td>
<td>129200</td>
<td>unknown 3</td>
</tr>
<tr>
<td>7</td>
<td>45.445</td>
<td>129890</td>
<td>240424</td>
<td>naringenin</td>
</tr>
<tr>
<td>8</td>
<td>56.095</td>
<td>360575</td>
<td>322984</td>
<td>polyphenolic</td>
</tr>
</tbody>
</table>

Table 4-27. Retention times and areas of compounds found in microvin wines 2007. Peak of areas are statistically different at 95\%, \( p<0.001 \), sed 29645 \((n=3)\).
Based on a similar spectrum and retention time, peak 3 could be a type of cinnamate ester as caftaric acid, though the spectrum was not quite the same. This possible compound was greater in shell wines. That same peak was also similar to ferulic acid (a derivative of trans-cinnamic acid), though its retention time it was not so close.

Another interesting compound greater in shell wines would seem to be naringenin, a type of flavanone which was found to be similar to peak 12 due to its spectrum and retention time. This compound was also found in grapes under UV exposure, according to its spectral properties (Kolb et al., 2003). However, peak 13, which was greater in control wine, seemed to be a polyphenolic similar to catechin due to its spectrum, but it has a late retention time. Spectral analysis did not allow identification of a group of three unknown compounds, whose concentrations were mostly greater in shell wines.

In addition, several unknown phenolic compounds defined by diverse retention times varied by comparing both treatments at p-values<0.05 (Figure 4-37). However, some of them were statistically different between treatments using the standard error of differences of means (sed = 94,543), most being greater in control wines. Further research using HPLC-MS would be useful in characterising these compounds.

Figure 4-37. Phenolics in commercial wines 2007. Mean areas expressed as µV*sec are statistically significant at p<0.05 between treatments, but not at p<0.001 for all of compounds. Bars indicate standard errors of treatment means (n=3).
The results obtained for flavonols in shell wines are in agreement with previous studies, which were made under increases of light, though berry skins were not analysed in this trial. Price et al. (1995) reported that quercetin concentrations in wines paralleled the levels in clusters, being greater in wines from sun-exposed clusters with values up to 35.2mg/L, much higher than the values found in shell wines: around 6.4mg/L. Similarly, total flavonols were also enhanced in wine coming from grapes under greater light as promoted by reflective groundcover (Spratt et al., 2007). Increases in flavonol levels were also found in berries from vines growing over aluminised reflective mulch (Coventry et al., 2005). Crawford (2006) established that flavonols were greater in grapes from the upper shell area at the beginning of the ripening period, but decreasing later as did flavonols in the control, but faster. The resulting wines had a much lower flavonol concentrations compared to the grapes at harvest.

Flavonol biosynthesis is light-dependent (Downey et al., 2004a) and the compounds are involved in UV screening due to their strong absorbance in UV (A and B wavelengths) and their accumulation in response to this radiation and sunlight (Price et al., 1995; Haselgrove et al., 2000; Spayd et al., 2002; Kolb et al., 2003; Pereira et al., 2006). Higher UV radiation increased flavonol content in ripening berries (Keller and Torrez-Martinez, 2004). So, clearly the higher amount of quercetin in shell microvin wines could be associated to increases of UV radiation and PAR.

Furthermore, quercetin has been also found as rutin or quercetin 3-O-rhamnoglucoside in grapes and wines (Monagas et al., 2005), which was similar between treatments with values of 0.77mg/L for shell and 0.65mg/L for control, though sometimes it has been confused with quercetin-3-O-glucuronide (Betés-Saura et al., 1996). Flavonols are present in wines as 3-glycosides and free aglycones, contrary to grapes, which only contain the glycoside forms due to acid hydrolysis that occurs during winemaking and aging (Monagas et al., 2005).

Higher flavonols found in shell microvin wines in 2006/2007 could be related to a greater perception of bitterness found for both shell commercial wines 2004 and shell microvin wines 2005 during the sensory evaluation. In addition, flavonols could have an impact on wine colour due to its yellow colour (though in red wines these are probably masked by anthocyanins, Ribéreau-Gayon et al., 2006b) and as an effective co-pigment for anthocyanins, mainly quercetin glycosides and cinnamic acids (Boulton, 2001).
However, when total pigments were analysed in wines vintage 2006, they were not changed.

Hydroxylated stilbenes have been cited as phytoalexins synthesized in skins in response to fungal infection (Dercks et al., 1995), and UV radiation, with resveratrol production being negatively correlated to berry development (Creasy and Coffee, 1988; Jeandet et al., 1991). The greater level of resveratrol in shell microvin (about 1.24mg/L versus 0.7 mg/L for control) could be explained as a positive effect of UV radiation, which was significantly higher in shell area compared to the control (see Figure 4-20, section 4.3.3). Threlfall et al. (1999) reported that grapes exposed to artificial UV light and then processed into wines had higher resveratrol levels, with values around 0.4 and 0.13mg/L for Cynthiana and Noble wine, respectively. However, not only there is a positive response of resveratrol production to UV radiation, but also sunlight, which was also greater in shell treatment by almost 9 times. While Price et al. (1995) reported similar results regarding resveratrol in exposed grape skin, levels of stilbenes were not affected by increasing light through a reflective groundcover (Spratt et al., 2007). In addition, it has been also confirmed that resveratrol level is variety-dependent, with Pinot noir one of the varieties with the highest content of this compound (Goldberg et al., 1995).

The amount of resveratrol extracted from skins during fermentation depends on the oenological conditions, especially in relation to increases in alcohol (Threlfall et al., 1999). Greater resveratrol concentration found in commercial control wine compared to microvin control (2.3 versus 0.7mg/L) could be related to some effect of the winemaking conditions during fermentation (and aging), though the variation between shell commercial and shell microvin was minimal (1.32 versus 1.24mg/L). Cold maceration before alcoholic fermentation facilitated stilbene extraction and $B$-glucosidase activity of Oenococcus oeni increased resveratrol content after malolactic fermentation (Poussier et al., 2003). Conversely in the shell trial, both commercial wines 2007 went through wild spring malolactic fermentation and they had not completed this fermentation when were analysed by HPLC. In fact, the control wine completed on 5th October, and shell wine is still (as of late November) going through the malo-fermentation.

Despite the fact that flavanols were quite similar between treatments in microvin wines, the amounts of these compounds were considerably higher in commercial control wines. Free monomeric forms of catechin and epicatechin were found to be lower in shell than
control samples for both microvinifications as well as commercial wines, being 7% and 10% lower in microvins, and 20% and 8% lower in commercial-scale wines, respectively. These results are in agreement with the findings of Crawford (2006) during the 2004/2005 season, also showing lower values in the shell treatment, and Price et al. (1995), who found greater concentrations of catechin and epicatechin in wine from shaded clusters, possibly originating in the grape seeds, though seed and skin catechin levels were not measured in this study. In the same way, no effect of increases of light on flavan-3-ols was found in wines and grapes by using reflective groundcover (Spratt et al., 2007). In addition, the values of catechin were higher than epicatechin in both microvin and commercial wines, which is similar to previous findings (Monagas et al., 2003).

Environmental factors such as heat and sunlight (Pastor del Rio and Kennedy, 2006; Cortell and Kennedy, 2006), even climatic conditions (De Beer et al., 2006) and altitude (Mateus et al., 2001) have been cited as affecting proanthocyanidin quantity and composition. Grapes in the control treatment received substantially less light and UV radiation compared to the shell area (see section 4.3), which explains greater proanthocyanidin values in control wines. However, increases in GDD (growing degree days) between fruit set and veraison in different seasons were associated with higher proanthocyanidin content in grapes and wines (Pastor del Rio and Kennedy, 2006). Only small differences were found in GDD calculated from flowering to veraison between shell and control areas (see section 4.3), and so this probably did not affect the proanthocyanidin concentration in the resulting wines.

Pastor del Rio and Kennedy (2006) also showed that grape maturity had no effect on the proanthocyanidin concentration in wine in comparing two seasons, though monomer flavanols increased with maturity in just one of the years studied. However, although flavones monomers are mainly seed-derived, proanthocyanidin differences in seeds were not correlated with flavan-3-ols monomer differences. On the other hand, while dimer and trimer flavanols reached higher concentrations in wines from riper grapes, indicating a greater degree of flavanol polymerization in riper grapes, monomers decreased, though epicatechin did not show this trend (Pérez-Magariño and González-San José, 2004). These results suggested that there are other factors influencing wine proanthocyanidin concentration.
It is possible that the greater flavanol concentrations obtained in the commercial scale wines are related to the differences in the winemaking conditions developed in both cases. Commercial fermentation conditions for both shell and control treatments were different to those used in microvinifications. Wild yeast and additions of pectolytic enzymes after pressing were utilized in the commercial fermentation. Sacchi et al. (2005) mentioned an increase of total phenolics and tannins by using pectinases, but no consistent results about the effect of yeast selection on the phenolic profile in red wines were reported. In the present study different times of maceration were applied previous to fermentation (4 days for control and 1 day for shell wines), providing the idea that flavanols of seeds (Sun et al., 1999) or from skin (Watson et al., 1995) could contribute to the proanthocyanidin concentration into the wines. Further research in seed flavanol content could reveal interesting information about this. In addition, commercial-scale wines were made under higher temperatures than microvins, consequently a greater phenolic extraction could be expected (Sacchi et al., 2005). Additionally, commercial wine samples were analysed were about 2 months in the barrels. It is well known that proanthocyanidins concentration in wines is determined by grape proanthocyanidin content, mainly in skins, seeds and stems, and by winemaking techniques and aging conditions (Ricardo da Silva et al., 1992; Gómez-Cordovés and González-San José, 1995; Fuleki and Ricardo da Silva, 1997). However, it was reported that while monomeric flavanols such as catechin and epicatechin decreased, trimeric and tetrameric derivatives increased during aging for 18 months (12 months in barrel and 6 months in bottle), demonstrating a greater polymerization and condensation of phenolic compounds (Pérez-Magariño and González-San José, 2004). In consequence, it seems to be that there is an effect of fermentation condition rather than aging in the commercial wines because greater monomeric flavanols were found in these wines, though flavanols and their derivatives were not measured by HPLC-MS as it was assessed by Pérez-Magariño and González-San José (2004).

Both conversion of tannins to oligomeric tannin-anthocyanin co-polymers, which stabilise wine colour (Peng et al., 2002), and the formation of low molecular weight phenolics during wine aging, reduce astringency (Cheynier et al., 2006). Sacchi et al. (2005) cited higher polymeric pigment concentrations when the fermentation temperature was increased, suggesting also the effect of winemaking techniques. Further analysis of the phenolic evolution during aging of shell and control wines could clarify the sensory perception differences.
Usually ripe grapes are associated with improved astringency quality, so an increase of skin proanthocyanidins in ripe grapes, improving astringency quality in the wines would be expected. However, wines made with riper grapes were higher in seed proanthocyanidins (Pastor del Rio and Kennedy, 2006). Better mouthfeel characteristics may be further related to the effect of soluble polysaccharides produced during fruit ripening (Vidal et al., 2004). Shell mulch modified the total sugar in grapes slightly, so a sensorial change could be expected in the resulting wines. In fact, Shell wines were lower than 2 g/L of residual sugar.

Gallic acid is the only hydroxybenzoic acid identified in seeds and skins occurring as a free form or flavanol ester (Monagas et al., 2005). While no effect has been reported of reflective light on hydroxybenzoic acids in berry skin as well as wines (Spratt et al., 2007), Crawford (2006) reported a small increase of gallic acid in shell grapes at the end of the ripening period and slightly higher amounts of this compound in shell microvin wines in 2004/2005 season. However, despite gallic acid being also slightly greater in shell microvin wine during 2006/2007 season (22.3 for control and 25.6mg/L for shell), this compound was lower in shell wines compared to control under commercial-scale, though having higher values than microvin wines (see Figure 4-36). Both commercial wines were in barrels some months before being analysed by HPLC. Greater amounts of hydroxybenzoic acid derivatives were found in wines aged in oak (McDonald et al., 1998). However, this does not agree with other studies where wines aged in oak contained lower free gallic acid levels, especially during the first month of aging (Pérez-Magariño and González-San José, 2005). Furthermore, greater gallic acid concentrations could be found in wine made by increasing skin contact time, suggesting an effect of maceration (Auw et al., 1996). In fact, both pre and post fermentation maceration were used in commercial wines (shell and control).

Caffeic acid, a hydroxycinnamic acid, was also slightly higher in shell wines. These compounds are located in vacuoles of skin and pulp cells as tartaric esters (Monagas et al., 2005) and their presence in wines is thought to be related to enzymatic hydrolysis of hydroxycinnamoyl esters (Price et al., 1995). A positive correlation of these compounds has been reported between grapes and wines. Caffeic acid, which was higher in shell microvin wines in this trial, was also greater in wine from sun-exposed clusters apparently due to faster hydrolysis of the tartaric acid moiety (Price et al., 1995) suggesting this effect on Shell wines. In the same way, total hydroxycinnamic acid levels
were higher in wines made from grapes exposed to reflective groundcover installed during berry development and enhancing light by 1.5 times in the fruiting zone (Spratt et al., 2007). Thus, increases of this compound under the effect of visible light and UV exposure together in the shell treatment could occur. However, UV had no effect on hydroxyl-cinnamic acids in grapes (Keller and Torrez-Martinez, 2004) and they decreased during acclimation to UV radiation, where flavonol formation was promoted (Kolb et al., 2003).

Caftaric acid, which was tentatively identified as being higher in 2007 shell microvin wines, responded positively in other studies, being greater in the shell area (Crawford, 2006) and in exposed grapes compared to shaded skins (Price et al., 1995). However, Price et al. (1995) showed no correlation for caftaric acid between wine and grapes, being lower in wines from sun-exposed clusters and higher in grape skins under same exposure. No differences were observed in wines made from grapes at different stages of ripening (Pérez-Magariño and González-San José, 2005). Both caffeic and caftaric acid, in a group together with benzoic acids (gallic acid), could have a sensory impact on wine due to synergistic effect of such mixtures lowering the taste threshold (Gawel, 1998), though Vérette et al. (1988) showed that none of these compounds by themselves were bitter in white wine.

Regarding coumaric acid, there was a study that compared wines made with grapes harvested at different stages of ripening, and there was not a clear trend in the levels of coumaric acid derivatives with harvest date (Pérez-Magariño and González-San José, 2004). This agrees with no differences found between treatments in the 2007 microvin wines.

In effect, although total phenolics were not different between shell and control wines measured at vintage 2005/2006 (see section 4.7.1) in this trial, by analysing individual phenolics it is possible to suggest that both wines were very different, which would contribute to the variation of several sensorial attributes in wines such as colour, body, bitterness and astringency, and producing changes in the volatile compounds contained in both wines.
4.7.3 Sensory Evaluation

Figure 4-38 and 4-39 show the distances of different characteristics rated in wines for each vintage and treatment, comparing commercial and microvin wines. The 2004 shell commercial wine evaluated in January 2006 was greater for all characteristics that define more complexity, such as total phenolics and phenolic ripeness, colour density, ripe fruit, bitterness, palate texture and overall quality. Hue was categorised as being similar between treatments. Crawford (2006) had reported lower colour and higher overall balance in Shell wines during a workshop tasting of the same wines in January 2005.

![Figure 4-38](image.jpg)

On the other hand, the same 2004 commercial wines had been assessed in January 2005 for the same volunteer panel, and most participants had preferred the 2004 commercial control wines, having more complexity, aroma and less green and unripe tannins (Crawford, 2006), contrary to the second wine tasting made in 2006. These differences found in the same wine during two followed years would be associated to changes in phenolic and aroma composition that wine goes through during the ageing process. Several reactions such as polymerization and condensation of phenolic compounds were reported in wines aged for 12 months in barrels and 6 months in bottles (Pérez-Magariño and González-San José, 2004). These reactions in wines would depend on the initial anthocyanin to tannin ratio and could influence astringency changes during ageing.
(Fulcrand et al., 1996). However, changes of flavour tend to be relatively limited during aging, meaning that a wine which tastes hard and astringent at the time of bottling, it will retain that character even after several years (Ribéreau-Gayon et al., 2006b).

![Figure 4-39. Mean sensory ratings for microvin wines (2005 vintage), evaluated in January 2006. Least significant differences (LSD) at p<0.001 for all of parameters. Standard error = 0.394 of treatments means (n=41).]

In looking at microvin wines 2005, while values for total phenolics and phenolic ripeness were clearly higher in shell microvin, hue and colour density resulted to be greater as well, but slightly compared to the control. However, other characteristics such as ripe fruit, palate texture, overall quality and bitterness were slightly greater in control wines.

Informal tasting of wines made in previous seasons have indicated that there is a trend of slightly riper fruit characters and greater elegance in the Shells treatment wines in comparison with the control (Crawford, 2006). Although any extrapolation of results reported by Crawford (2006) are limited due to the difference in seasons, the majority of participants have preferred the shell microvin wine 2004, perceiving it as being greater in surface smoothness, complexity, texture and heat (Creasy et al., 2006). This microvin wine was also perceived to be harsher than control (Crawford, 2006), which agrees with findings of Price et al. (1995), who reported harsher wines and higher levels of flavonols from sun-exposed clusters. It seems to be that a greater grape maturity in shell area would be affecting positively the flavour and sensory profile of the wine, not only in mouth feel characteristics, but also in aroma compounds.
Astringency and bitterness in red wines are influenced by proanthocyanidin content (Gawel, 1998). While the perception of bitterness is restricted to small molecules with particular structural features, astringency is related to the precipitation of salivary proteins which provide lubrication in the mouth, and its perception in wines depends particularly on tannins extracted from skin and seeds during alcohol fermentation (Cheynier et al., 2006). Thus, decreases of bitterness and increases of astringency with higher flavanol sizes (Noble, 1998) and greater proanthocyanidin degree of polymerization (Cheynier et al., 2006) are expected during fermentation. This could be correlated to microvin as well as commercial control wines, with vintage 2004 and 2005 being less bitter and more astringent (less phenolic ripeness) compared to shell ones due to higher amounts of monomer flavanols such as catechin and epicatechin obtained in wines vintage 2007 (see Figure 4-38 and 4-39, section 4.7.3). Shell wines from vintage 2004 were perceived to have less bitterness than control wines (Crawford, 2006).

4.7.4 Gas Chromatography – Mass Spectrometry analysis

Two microvin wine samples of 2007 vintage (shell and control) without replicates were analysed using GC-MS through the Solid Phase-Extraction (SPME) by Simpson (2007). Although no statistical comparison was possible, some trends about aroma compounds in wines by comparing shell and control areas were identified. Although chromatograms revealed that volatile components were greater in the control sample in number of compounds and total area obtained, the distribution of aroma compounds was different. Hence, while the number of identified aldehydes and esters were greater in the control wine, other compounds such as acids, alcohols and hydrocarbons were higher in shell wine. The number of lactones and ketones were similar for both samples.

However, the relative area proportion of compound classes such as acids, esters, hydrocarbons, ketones and lactones was greater in shell wine, whereas alcohols, aldehydes and undetermined compounds were higher in control wine.

It has been cited that Pinot noir aroma is a complex of different compounds rather than a single compound responsible for the characteristic aroma (Fang and Qian, 2006). In fact, from a preliminary report it was concluded that red and dark fruit sensory profile, slightly sweet and high alcohol content predominate in a high quality Pinot noir wine (Guinard and Tsay, 2007). Acids and higher alcohols are formed during fermentation and they
have high sensory thresholds, being less important to wine aroma (Fang and Qian, 2005). Esters should contribute to the characteristic fruity aromas of the wine because its sensory thresholds are at low levels expressed in µg/L (Fang and Qian, 2006).

By looking at specific odour-active compounds (Simpson, 2007), the peak area of geraniol, an important monoterpene alcohol that contributes floral and cherry flavours to Pinot noir wines (Fang and Qian, 2005), was greater in shell wine. Terpene alcohols increased in Pinot noir wines when grapes were harvested at higher sugar content (over 25° brix), presenting more floral aromas (Fang and Qian, 2006). In addition, linalool could be transformed to geraniol during winemaking processes through enzymatic reactions (Hernandez et al., 2003). However, 1-hexanol, which contributes fruity aromas related to grape juice (Fang and Qian, 2005), was lower in shell wine.

β-Damascenone, which is variably described as having apple, rose and honey aromas, was detected in shell wine. C13 norisoprenoid precursors come from carotenoid degradation during grape ripening (Razungles et al., 1993; Bureau et al., 1998; Ebeler, 2001) and they increase with grape maturity (Fang and Qian, 2006), mainly under UV-B exposed conditions (Schultz, 2000), which could explain this result. However, this compound was greater in Riesling wine made from fruit protected against UV radiation (Lafontaine et al., 2005) and when no leaves were removed in Cabernet Sauvignon (Lee et al., 2007).

Peak areas obtained for fatty acid esters such as ethyl butanoate, ethyl hexanoate, ethyl decanoate, and ethyl octanoate, were higher in the control wine. Esters are considered secondary aromas which supply fruity odours, and though they are affected by yeast strain, temperature, oxygen and nitrogen levels during fermentation (Clarke and Bakker, 2004; Beltran et al., 2005), their concentration decreased with grape maturity (Fang and Qian, 2006). However, the contribution to wine aroma could be restricted due to its high detection threshold (Fang and Qian, 2005). Another ester also contributing to fruity aromas, but reported as less important in Burgundian Pinot noir (Moio and Etievant, 1995), was ethyl dihydrocinnamate, which was identified as being greater in shell wine.

With regard to acids, butanoic acid, which imparts sweaty odours, was not found in control wine. Propanoic acid, which is related to spicy aromas, was higher in control
wine, and both hexanoic and octanoic acids were greater in shell wine, possibly contributing to sweaty and goaty rancid cheese aromas (Fang and Qian, 2005).

In consequence, the concentration of aroma compounds and their balance within wine would affect the quality of Pinot noir wines, and it seems to be that aroma active compounds increase along with grape ripening, a stage where shell mulch is modifying the light environment (PAR and UV radiation) rather than canopy temperature or GDD. In fact, it has been demonstrated that using white geotextile reflective mulches may improve sunlight exposure and reduce herbaceous aromas in Cabernet franc (Hostetler et al., 2006). Further research needs to be done to understand the effect of light and temperature on grape maturity and aroma composition. However, these preliminary trends agree with a study that identified aroma compounds in Pinot noir wine from Oregon (Fang and Qian, 2005), where a wine described as spicy, vegetative and floral was greater in compounds such as propanoic acids and aldehydes, whereas a fruity wine was related to higher quantities of esters, which is similar to what was found in shell wine components.
CHAPTER V
CONCLUSIONS

The research was run over four seasons to evaluate the effect of using mussel shells as reflective mulch on vine performance and fruit and wine quality. However, new contributions of the last season (2006/2007) are presented in conjunction with the confirmation of others results. Shell mulch had several effects on phenological growth stages in grapes and also on sensory perception of the wine due to modification of phenolic and aroma compounds. The findings may be divided into several categories:

5.1 Vine Environment:
Both the light environment inside the canopy and soil temperature was markedly different between treatments. It was confirmed that the soil under mulch was cooler compared to un-mulched control, but shells buffered extremes in temperatures. Crawford (2006) had reported fewer weeds in Shell area. However, Shell mulch reduced weed growth compared to control after measuring coverage. Species were also identified, being Malva sp. the most frequent.

This trial confirmed that fruiting zone temperature over shells was slightly higher during the day and cooler at night. However, growing degree days and temperatures were not modified significantly as a result of shells. Shell mulch reflected greater amounts of UV-A, UV-B and PAR radiation into the fruiting zone as well.

5.2 Vine phenological stages:
Dates of flowering and veraison appeared to be slightly advanced over shells. Peduncle lignification was delayed at veraison as well as at harvest time in the shell mulch area. Budburst does not seem to be affected by shells and there was not a clear effect on advancement of harvest date. The influence of seasonal weather conditions seems larger than the effect of treatment. Fruit set was similar between treatments but was considered poorer in shell bunches due to large population of seedless berries during this season. The large number of shot berries may be driving other differences observed especially grape composition.

Vine growth was not affected by shells in terms of the number of nodes laid at pruning, cluster and shoot number pre shoot thinning, and so did not have an impact on yield.
Early shoot growth, shoot lengths, pruning weights, trunk circumferences and canopy density were similar between shell and control, though internode lengths were more associated to differences in seasonal weather conditions. In consequence, vine vigour as measured through these variables did not increase with use of shells.

Nutritional status was affected by shell treatment. Leaf petiole and blade samples showed slightly higher amounts of calcium in shell compared to control. However, levels of Na, Zn, Bo and Mo were the highest increases in Shell area.

Leaf chlorophyll content measured as SPAD values were higher in the shell treatment during veraison, pre- and post-harvest, but lower shortly after budburst.

Yield components were affected by shell mulch, but depending on the seasonal conditions. While berry weights were higher in shell clusters during 2005/2006 season, bunch weights and yields were lower in shell than control during 2006/2007 season.

5.3 Juice and wine:
There were slight differences between treatments in fruit and wine composition of variables such as °Brix, TA, pH, alcohol. While °Brix and pH were similar, only TA was higher at harvest. However, shell must after crushing was greater in °Brix but similar to control for pH and TA.

HPLC analyses of commercial and microvin wines showed preliminary differences of the individual flavonoid composition. While shell microvin wines showed greater quercetin and resveratrol concentrations than control, commercial shell wines were lower in epicatechin, gallic acid, resveratrol, and catechin than control. However, no differences in total anthocyanins and total phenolics between treatments were found by spectrophotometer.

Leaf phenolic composition was also different between treatments, demonstrating a possible effect of increased UV radiation and PAR. However, identification of individual compounds was not within the scope of this study.
Sensory analyses of microvin and commercial wines showed some differences between treatments, exhibiting lower levels of green and unripe tannins, and greater smoothness and complexity as well.

Despite in the fact that past research using reflective mulches have shown some effects on viticultural aspects and grape and wine composition, just few works could related some of them to light. Even, mostly works have assumed influences of light and canopy temperature on vine performance and wine quality. However, this new research has contributed to a greater understanding of which variables are influencing changes in grape development and wine composition. Particularly, light consisting in PAR and UV radiation rather than temperature modified the vine environment affecting some particular growth stages such as flowering and veraison, providing changes in berry composition. So resulting wines were improved in sensory characteristics related some perceptions to different content of phenolic compounds.
CHAPTER VI
FURTHER RESEARCH

Noticeably, the use of reflective mulch had important effect on grape as well as wine composition. However, some of the findings proposed by this research have not been clarified yet. Malic acid concentration was found higher in shell grapes at harvest contrary to previous studies which indicates that malate decreases during ripening period due to the effect of temperature on the balance between malic acid synthesis and catabolism. Shells influenced the grape environment throughout the whole season and could cause changes in metabolic processes in grapes which occur during berry ripening. This development would be related to rates of photosynthesis, but it needs to be investigated.

Further analyses by HPLC-MS in wines, leaves and grapes at harvest are necessary to identify individual phenolic compounds that were affected by reflective mulch. PAR and UV radiation could modify the light environment, but not temperatures. At the same time, collections of fruit samples at different stages during ripening period for phenolic analyses could be related to resulting wines clarifying the effect of light and UV radiation on anthocyanins, flavonols, flavanols and stilbenes.

Sensory analyses of microvin and commercial wines were consistently different between treatments, exhibiting changes in the wine perception. Further analysis by GC-MS and HPLC-MS are essential to recognize in particular which compounds are affected and the relationship that they have with other phenolic compounds at different phenological stages not only in skin and seeds, but also in resulting wine.

These new objectives could give a better understanding of the phenomenon that is happening under the influence of increased light in the fruiting zone.
ACKNOWLEDGEMENTS

I would like to thank to my supervisor, Dr Glen Creasy, for his support, encouragement and time spent on my thesis. He was the person who gave me the opportunity to come to New Zealand and to involve me in an important research project for the wine industry. Thank you to Dr Roland Harrison, my co-supervisor for his time spent reading and critiquing my writing. Thank you to Richard Hider for assistance in the HPLC analyses.

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Finally, I would want to thank to my family for their eternal interest and enthusiasm from far away. Especially, my son Gerardo Jr., Dad and sister.
REFERENCES


Fang, Y. and Qian, M.C. (2006). Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. *Journal of agriculture and food chemistry* 54 (22): 8567-8573.


APPENDICES

Appendix A: Pinot noir Workshop - Tasting Sheets

The Mouthfeel Wheel


The Process

You will be presented with three wines, each with a number assigned to it.

Please fill out following page while tasting the wines. For the Appearance, Aroma, Mouthfeel and Overall Balance categories, please answer by drawing a line where you perceive to sit on the relative scale, and then mark that line with the wine's code. Please ask if you are uncertain as to what to do.

Your responses will be collated and a summary returned to Nelson Grapegrowers and Winemakers.

Example

Expected suitability for Robert Parker

Low

673

422

095

High
Appearance
Low
High

Color

Purple |

Red |

Brown |

Hue

Aroma
Low
High

Ripe fruit character

Mouthfeel
Short
Long

Palate length

Low
High

Bitterness

Tannins
For this section, pick the 5 best descriptors for the tannins in each wine, using the Mouthfeel Wheel on the previous page:

Wine 1:
Wine 2:
Wine 3:
Wine 4:

Overall Balance
Poor
Excellent
Appendix B: Correlation between °Brix and berry weight at harvest 2006 (Control and shell).
Appendix C: Differences of canopy temperatures postharvest (from 1st April to 26th July).

Appendix D: Soil temperatures post harvest, April to July 2007.
Appendix E: Chromatography of phenolics compounds. Chromatogram shows absorbance versus retention times of phenolics from microvin control wine 2007. Peaks show specific retention times at which were statistically different.

Appendix F: Chromatography of phenolics compounds. Chromatogram shows absorbance versus retention times of phenolics from microvin shell wine 2007. Peaks show specific retention times at which were statistically different.
Appendix F: Grape nutrition report, December 2006.

GRAPE NUTRITION REPORT
December 2006
Neudorf Vineyards
Blade and Petiole Analyses
Toms Block

Content of report:
- Introduction
- Comments on blade and petiole analyses
- Advice to improve nutrient balance for Toms Block vines
- Analyses results
Introduction

Interpretation of the results of four blade and four petiole analysis received from Hill Laboratories. The Good and Affected areas from the bottom area of Toms Block were taken by Sjef Lamers. The Shells and Control samples were taken by a Lincoln University student for the mussel shell trial.

Nutrient uptake of vines varies based on rootstock, varieties (and clones), soils, moisture availability, environmental circumstances like weather (frosts, rain and strong winds) and cropping history. Nutrient concentrations differ based on leaf age, leaf position on the vine, time of the day, etc.

Disclaimer: The opinions expressed in this report are based on the analysis results as received, and information made available and/or known at this time to us and all due care was exercised in its preparation; no responsibility can be accepted for the outcomes, or events beyond our control. As the mixing and application of fertilizers and chemicals is outside our influence and control we cannot take liability for any damage. Any subsequent action in reliance on the accuracy of this information is the sole commercial decision of the user of the information and is taken at his or her own risk.

Comments on leaf and petiole analysis

Field visits on 1 and 5 December 2006 were conducted to observe, discuss and take samples from vines displaying stress symptoms. Several rows were affected, some in the main vineyard and some in the bottom area of Toms block. During the 5 December visit I noticed that the top area of Toms Block was also affected. Some shoots showed cupping of leaves, misshapen leaves and necrosis. The most affected area were some rows in the bottom area of Toms block.

Several elements show large differences between the Good and the Affected vines (see table 1).

<table>
<thead>
<tr>
<th></th>
<th>Blade Good</th>
<th>Affected</th>
<th>Petiole Good</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>1.0</td>
<td>0.7</td>
<td>1.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>330</td>
<td>970</td>
<td>150</td>
<td>220</td>
</tr>
<tr>
<td>Boron</td>
<td>48</td>
<td>174</td>
<td>41</td>
<td>46</td>
</tr>
</tbody>
</table>
The analysis results point to three elements being very high:

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Tom’s Bottom area</th>
<th>Bottom and Top of Tom’s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Affected</td>
</tr>
<tr>
<td>Manganese</td>
<td>Bla</td>
<td>Pet</td>
</tr>
<tr>
<td>1000</td>
<td>920</td>
<td>250</td>
</tr>
<tr>
<td>Zinc</td>
<td>330</td>
<td>970</td>
</tr>
<tr>
<td>Boron</td>
<td>48</td>
<td>174</td>
</tr>
</tbody>
</table>

Papers in the Proceedings of the 2004 ASEV conference on grape nutrition (e.g. Robinson – Application of Nutritional Standards) mention boron excess or toxicity as more than 100 ppm in the petioles or in the blade a level higher than 150 ppm (quoting Robinson et al 1997). The same paper (Robinson 2004) mentions a level of >250 ppm for boron excess at veraison according to Weir&Cresswell 1993. The blade is seen as more reliable for confirmation of boron excess or toxicity. Christensen in the paper “Foliar Fertilisation in Vine Mineral Nutrient Management” of the same conference mentions that spring and summer foliar application should not exceed 0.55 kg B/ha for each spray in order to avoid phototoxicity.

Excessive or toxic levels of manganese and zinc were not mentioned. Such high levels can be due to:

- plants taking up excess manganese during adverse climatic conditions
- acid soil conditions,
- fungicide sprays.

The very high concentrations would increase phototoxicity/leafburn potential.

The slow growing affected vines of Toms Bottom area would get higher spray concentrations than the faster growing vines. The faster growing vines can dilute the concentrations by having more leaves (more biomass).

Potassium is marginal to low in most petioles and blades except Toms Bottom Good petiole sample. It seems that the leafburn has had an effect on the potassium levels in the tissues as can be seen from the blade and petiole analyses from Tom’s bottom area in Table 1. The Good blade and petioles have higher potassium levels than the Affected blade and petioles.

The molybdenum is low in the petioles of Tom’s Bottom area and marginal in the petioles of the Shells and Control. The blades have reasonable levels in the Shells and Control and low in Tom’s Bottom area. It seems that Tom’s Bottom would require
attention for molybdenum. Molybdenum availability in the soil is linked to pH level: low pH results in low availability.

The very high manganese levels in Tom’s Bottom can also point to acid soils. I suggest to take top and sub soil samples in this area to determine if the roots are in acid soil horizons.

The Shell trial shows a strong increase in calcium in blades and petioles compared to the control, while Tom’s Bottom area has lower calcium levels then the Control.

Table 3: Calcium levels:

<table>
<thead>
<tr>
<th></th>
<th>Blades</th>
<th>Petioles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shells</td>
<td>2.19</td>
<td>2.17</td>
</tr>
<tr>
<td>Control</td>
<td>1.58</td>
<td>1.45</td>
</tr>
<tr>
<td>Tom’s Bottom</td>
<td>1.26</td>
<td>1.12-1.30</td>
</tr>
</tbody>
</table>

Normal 1.2-2.5 1.2-2.8 (Robinson)
NZ average 1.2-2 1.3-2.1 (average of Hill data)

The calcium level of Tom’s Bottom is in the lower part of the normal range with one petiole level (from Good sample) dropping into the low range; this is another reason to check the acidity of the sub soil.

Advice to improve nutrient balance for vines

Based on the analyses results I suggest to apply the following fertilisers and to keep spraying at fortnightly to three week intervals till mid veraison.

1. Foliar sprays till mid veraison at intervals of 14-21 days at a rate of 500-600 liters of water per hectare:
   - Solupotasse (potsulfate) 1000 grams/ha
   - General foliar feed 4 liters/ha (e.g. Librel MGF at 2.5 kg/ha, Tracel at 4 Ltrs/ha, Filocal 360Winegrape at 4 Ltrs/ha, Liqui-Trace at 3 ltr/ha or Wuxal MicroPlant at 1 Ltr/ha)

Please take the normal precautions like follow manufacturer’s recommendations and spray in early morning or during overcast conditions to prevent leaf burn.
Librel MGF (Micronised Grape Feed) is available from Tasman Crop Protection, Filocal
360Winegrape is available from Appleman Pons in Motueka (fax 03 5288216), Liqui-
Trace is available from Ravensdown.

Please take the normal precautions like follow manufacturer’s recommendations and
spray in early morning, evening or during overcast conditions to prevent leaf burn. If
you like to combine with sulfur and fungicide sprays please check first if fertilizers are
compatible with chemicals.

Sometimes leafburn can occur or the fertilizers diminish the effectiveness of the
chemicals (via high or low pH). The total concentration should be less than 3% w/v of
fertilizers, fungicides and any other additions combined.

2. I suggest to take top and sub soil samples in autumn/winter.

Please contact us if you like to change the fertilizer mix or if you require any
further information or explanation.

Wakefield, 19 December 2006
Sustainable Nutrition

Sjef Lamers
B For Sc., Dip HLS Dordrecht, MNZSSS